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STAGONOSPORA LEAF SPOT AND ROOT ROT OF FORAGE LEGUMES[1]

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INTRODUCTION

A root rot of alfalfa (*Medicago sativa* L.) not previously described was found almost simultaneously at Riverside, Calif., and Madison, Wis. The causal agent has been shown to be a species of *Stagonospora* known previously only as a leaf-spotting fungus of several legumes. Since no leaf spot was found associated with the root rot, at least in California, a study was made to determine definitely the identity of this fungus with similar fungi on other closely related plants. In these investigations all available herbarium material of these fungi on all of the forage legumes in the genera *Medicago*, *Melilotus*, and *Trifolium* has been examined and cultural studies of living material have been made as far as possible.

An ascigerous stage belonging in the genus *Leptosphaeria* has been found on certain host species but not on all of them. For this reason the writers have chosen to designate the disease on all of the hosts as stagonospora leaf spot and root rot. A new combination is suggested for the *Stagonospora* on *Trifolium pratense*.

THE DISEASE

In the production of disease of possible economic importance within the observation of the writers, the species of *Stagonospora* with which this paper is chiefly concerned merits attention as a cause of a root rot of alfalfa and of a leaf spot and stem blight of sweetclover. The fungus has been found producing a seemingly unimportant leaf spot on other host species, and has been cultured from them, the complete list being as follows: *Medicago sativa* L., *M. lupulina* L., *M. arabica* Huds., *Melilotus alba* Desr., *M. officinalis* (L.) Lam., *M. dentata* (Waldst. and Kit.) Pers., *Trifolium repens* L., *T. hybridum* L. It has been seen in herbarium collections on *Trifolium alpestre* L. The leaf spot on all of these hosts is so similar that the description of it on alfalfa applies adequately to all.

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On alfalfa the leaf spot is a light buff, pale olive buff, to almost white (fig. 1, *A*). Many of the spots have a darker brown border—bister to sepia of Ridgway (*35*)[3]—between the gray center and the green healthy tissue. In some spots there are other, darker brown,

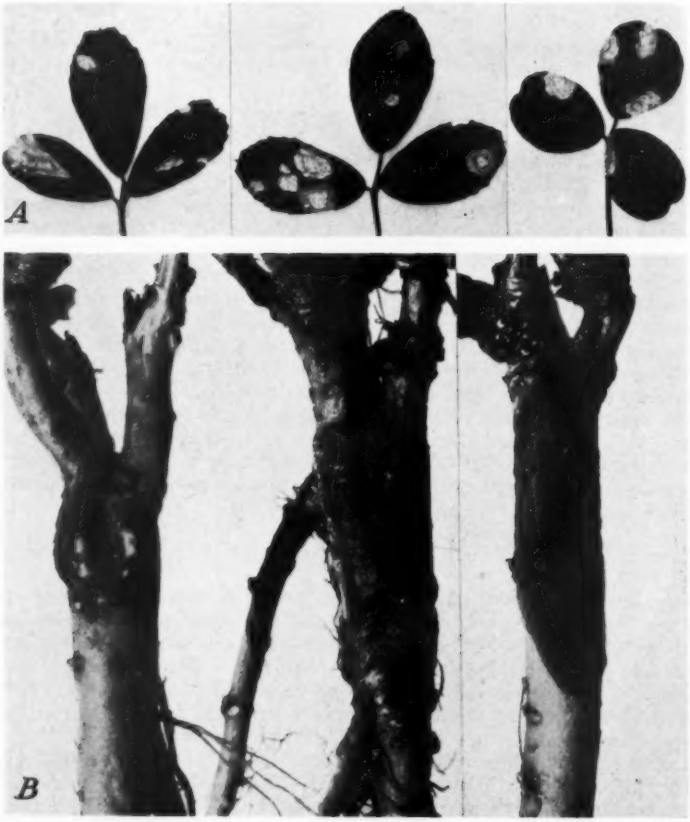

FIGURE 1.—Leaf spot and root rot of alfalfa produced by artificial inoculation with *Stagonospora meliloti* at Riverside, Calif. *A*, Leaf spot of alfalfa seedlings produced by inoculation. × 1¼. *B*, Root and crown rot of alfalfa produced by inoculation with *S. meliloti* from alfalfa. The wound on the uninoculated plant at the left is completely healed. Inoculation made August 4, 1931; photographed June 9, 1932. × about 1¼.

more or less concentric narrow bands in the central gray portion of the spot. The margins of the spots are sometimes water-soaked in appearance. In the light central portion are numerous light-brown to nearly black pycnidia. Pycnidia are sometimes developed near the margin of the spot when the foliage is kept at high humidity.

[3] Italic numbers in parentheses refer to Literature Cited, p. 810.

The spots range from 1 mm to 1 cm in diameter, or they may involve nearly the entire leaflet.

Stem blight was produced on alfalfa, both at Riverside and at Madison, but was not observed in the field. Ashy lesions bearing pycnidia often girdled the stems, causing the death of the shoot. Petioles were likewise attacked.

As a root rot of alfalfa, the disease occurred at Riverside only on the upper part of the taproot and on the crown branches (fig. 1, *B*). The surface of the lesion is dark brown to black in color, usually smooth at first, but becoming roughened as the outer layers dry and crack. Beneath the surface the tissues are reddish brown to almost black. They are moist at first but dry out later, forming a dry rot that may involve a considerable part of the root or crown. The taproot may be severed by a lesion. The disease progresses slowly, requiring several months in which to rot off a medium-sized alfalfa root. A characteristic reddish color is often present in the decaying wood rays through which the disease makes its way toward the center of the root. No new buds are formed above the lesion, and the plant eventually dies. Since the disease progresses slowly, secondary invaders enter the diseased tissue and undoubtedly contribute largely to the destruction of the plant.

As a root rot the disease was first found at Madison in the overgrowths of grafted plants transplanted to the field. Here the lesions strongly resembled crown rot caused by *Phytomonas insidiosa* McCulloch, from which the stagonospora root rot was first distinguished by microscopic examination. Later the stagonospora rot was found in plants inoculated with *P. insidiosa* for the purpose of testing their resistance. Finally *Stagonospora* was discovered causing a crown rot in a small percentage of plants in an alfalfa field. The most characteristic root rot lesions are wedge-shaped discolorations extending from the bark toward the center of the root. The crown rot is distinguished in the field only with difficulty, and thus an estimate of its importance in comparison with other diseases is not easily made.

On *Melilotus* spp. the leaf spot has usually been found most abundantly early in spring and late in the fall, on foliage produced in cool weather. However, on a variety of sweetclover with fine stems the leaf spot has been abundant in midsummer as well.

The fungus invades stems extensively on occasion, though for the most part so late in the fall that the damage is probably slight. Once the tops of young tender shoots in a dense growth were found killed by the fungus, which was fruiting abundantly. Only once has the *Stagonospora* stage been found fruiting abundantly on flowering stems, and in this case the plants had been checked by drought. Here the lesions resembled so closely those of a species of *Ascochyta* occurring on this plant that the fungus was recognized only after microscopic examination. In early autumn the bases of stems of plants of *Melilotus alba* are often invaded extensively by the fungus in a manner described later, with little external evidence beyond a paler green color and a slight browning of the interior tissue until the *Phoma* stage of the fungus begins to develop, when the color changes to a golden brown and the surface becomes finely dotted with the deeply seated fruiting bodies.

A root rot of sweetclover has been produced readily by inoculation, but has been found only once in the field. Lesions may be black in early stages, but when extensive are usually ash-colored and dry.

PATHOLOGICAL HISTOLOGY

At Madison the stagonospora root rot was first recognized and distinguished from bacterial wilt of alfalfa by the discovery in diseased tissue of blue-staining fungus threads where blue-staining bacteria were expected after the application of the Gram stain (*33, p. 17*). After some exploration it was found that the fungus mycelium has this unusual and most useful staining reaction only when growing in certain situations. In roots and in large stems on which the fungus has not fruited it stains well; in leaves and in culture media the small fragments of fungus thread that have been stained are so few that for practical purposes it may be said not to stain at all in those situations. Spores in pycnidia often stain brilliantly, both on the host plant and in culture. The staining of a fungus in its host plant with the Gram stain, as described above, has not been previously recorded so far as the writers are aware. Roots in which the stain revealed the fungus were cultured on many occasions, and in no instance did the fungus fail to grow. Finally the stain was used as a rapid and, it is believed, a reliable test for the invasion of the fungus both in field material and in inoculated roots when the lesions were of doubtful character.

The fungus appears to enter roots of alfalfa and sweetclover only as a wound parasite. The mycelium is easily stained and traced between the parenchymatous ray cells in approximately the same region often occupied by the bacteria causing wilt. However, the fungus develops more extensively between the larger cells at the center of the rays rather than between the smaller cells at the margins of the rays, where the bacteria are more frequently found, and thus it does not approach or enter the vessels. Mycelial strands are often found far in advance of discoloration in invaded tissue (fig. 2, *B*). Sometimes the older mycelium bears small knobs resembling haustoria, but their penetration of the host cell walls against which they are pressed has not been satisfactorily demonstrated. Overgrowths of any kind, where cells and intercellular spaces are large, appear to be particularly favorable regions for invasion (fig. 2, *A*).

PATHOGENICITY OF THE FUNGUS

INOCULATION EXPERIMENTS AT RIVERSIDE

Isolations were made from several diseased plants, and cultures of several species of fungi were obtained. On April 14, 1931, 5 of these species, including the species of *Stagonospora*, were each inoculated into 10 healthy alfalfa plants growing out of doors in 16-gallon garbage cans, and 10 plants were held as controls. The plants were about a year old, and the roots measured 8 to 10 mm in diameter near the wound. Hyphae from agar cultures were inserted into wounds made in the taproots just below the crown, while the controls were wounded but not inoculated. On June 9, 1932, the plants were removed from the soil and examined. Six of the plants inoculated with *Stagonospora*

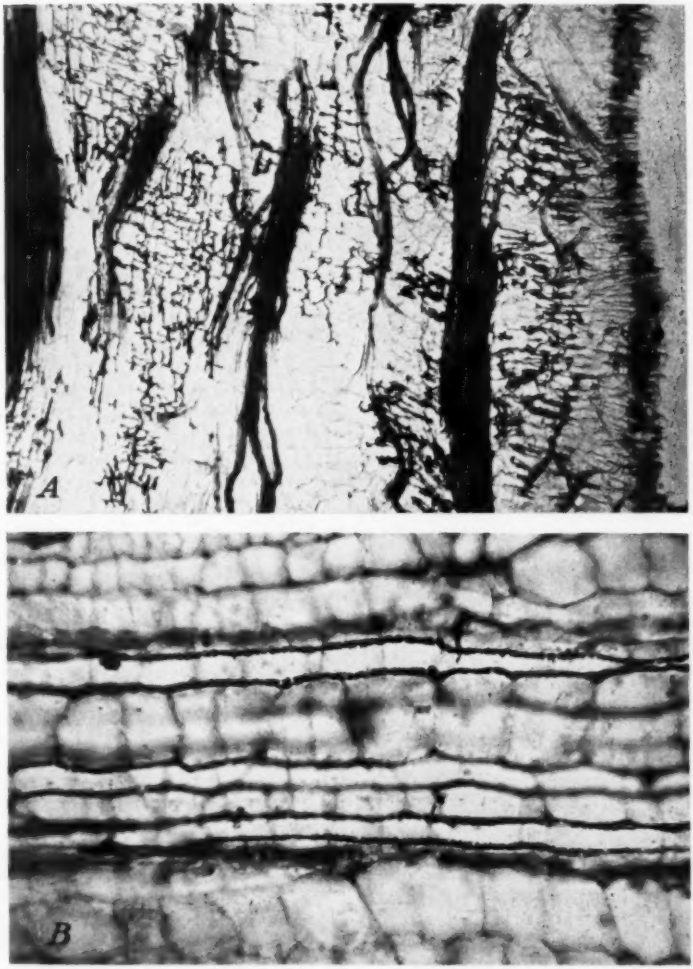

FIGURE 2.—Mycelium of *Stagonospora meliloti* stained in host tissue. *A*, Mycelium in a longitudinal section of the phloem of a grafted alfalfa plant. Slight discoloration of the living tissue indicated this diseased condition. The fine dark lines represent deeply stained intercellular mycelium. From natural infection. *B*, Mycelial strands of the same fungus passing through a wood ray of *Melilotus alba*, far in advance of discoloration. From artificial inoculation. × 450.

were more or less decayed about the wound. The other roots, including the controls and those inoculated with the other fungi, were healthy. Figure 1, *B*, shows two of the infected plants and one of the controls.

A second set of inoculations was made in January 1934. Alfalfa plants about a year old were inoculated and set in cans, which were held in soil-temperature tanks at five different temperatures, namely, 36°, 32°, 28°, 25.5°, and 19°–21° C. Ten plants, inoculated by inserting bits of hyphae from an agar culture into wounds, were held at each temperature. All of the wounds were covered with rubber electric tape. The temperature usually did not vary over 1° except in the 19°–21° tank, which varied as much as 2°. The experiment was discontinued on April 18, 1934. At no time were there any top symptoms that suggested root infection. When the roots were examined it was found that there were 0, 3, 10, 6, and 9 plants infected at 19°–21°, 25.5°, 28°, 32°, and 36° respectively. There was no infection in any of the controls. The lesions were all small, and it was evident that the experiment should have been continued several months longer. The fungus had spread into the tissues and formed dark-reddish islands or streaks, such as are characteristic of the decay in the deeper tissues of naturally infected plants. Isolations were made from a number of plants, with the following results: The fungus was recovered from eight of the plants infected at 36°, from four at 32°, and from seven at 28°.

On June 12, 1934, 7 lots of 25 healthy 1-year-old plants were inoculated with 7 isolates of the fungus respectively. The hyphae from agar slants were inserted beneath the bark through wounds which were then closed by being wrapped tightly with raffia. Twenty-five controls were prepared by wounding and wrapping. The plants were then set in an isolated plot of ground on which alfalfa had not been grown for several years, if ever. Thirty-five of the plants did not survive the transplanting. The number of healthy and diseased plants alive on October 11, 1934, as well as the percentage of infection, is shown in table 1. Of the inoculated plants alive on October 11, 1934, 49.3 percent were infected. Most of the lesions were small but were sufficiently far advanced to show their true nature. None of the controls was infected.

TABLE 1.—*Percentage of living plants diseased on October 11, 1934, out of 25 healthy 1-year-old alfalfa plants inoculated with different isolates of Stagonospora meliloti on June 12, 1934, at Riverside, Calif.*

Source of fungus	Healthy plants	Diseased plants	Infection
	Number	*Number*	*Percent*
(1) Natural infection, Riverside	16	5	23.81
(2) Do	13	3	18.75
(3) Artificial inoculation with No. 2	4	19	82.61
(4) Same as No. 3, but another isolation	11	11	50.00
(5) Same as 3 and 4, but another isolation	13	8	38.10
(6) Natural infection, Madison [1]	8	8	50.00
(7) Do.[1]	7	16	69.57
(8) Control	23	0	.00

[1] Cultures supplied by F. R. Jones.

Although there was no field evidence of the parasitism of this fungus on leaves, inoculations of foliage were made. On March 18, 1935, two 6-inch pots of alfalfa seedlings about 6 inches tall, grown from seed in the greenhouse and free from leaf diseases, were sprayed with a spore suspension. The plants were then held with controls for 3 days in an inoculation chamber where the humidity was high.

The time required for infection to appear was not determined, but on April 4 numerous more or less circular or rarely V-shaped spots were present on the leaves of many of the inoculated plants. A fungus which proved to be identical with that used in inoculation was isolated from several of the leaf lesions.

INOCULATION EXPERIMENTS AT MADISON

The inoculation trials at Madison were designed chiefly to determine whether the *Stagonospora* stage of the fungus passes readily from one host species to another. Foliage and root inoculations were made, chiefly on alfalfa and sweetclover with spores from cultures.

Foliage inoculations were largely unsuccessful. However, alfalfa was infected with cultures from alfalfa, and sweetclover was infected with some cultures from sweetclover. *Trifolium repens* was not infected with spores from any source. In the cross-inoculation trials the only success was a slight infection of white sweetclover foliage with one culture from alfalfa. Cultures from *Medicago lupulina* and *M. arabica* were not available when most of these tests were made, and therefore these have not been tested on foliage. In these inoculations, as in those at Riverside, it was at least 2 weeks after inoculation before spots appeared on the leaves. This long incubation period was unexpected, since the leaf spot occurs in the spring on leaves that can hardly be 2 weeks old. Moreover, with alfalfa the Peruvian and non-winter-hardy varieties were infected more readily than hardy Turkistan plants. Experience with these foliage inoculations suggested two things: (1) Differences in varietal susceptibility within a species might be greater than difference in susceptibility between species; (2) in the field another spore form in addition to conidia might be responsible for infection. The successful search for the perfect stage was in part inspired by the latter possibility. Since the perfect stage has been found in sufficient quantity for inoculation work only on sweetclover, few cross inoculations with it on foliage have been attempted. Thus far succcess has been obtained only in infecting foliage of sweetclover. No inoculations have been attempted with the *Phoma* stage.

Root inoculations with the fungus from various host sources were tried in the hope that these would furnish an easier test of the ability of the fungus to pass from one host to another. Sweetclover roots were used more extensively than those of alfalfa, since they could be stored more readily for use during the winter and could be sectioned more easily when microscopic examination of the lesions was necessary. As in the previously cited experiments at Riverside, rapid infection could be obtained only at moderately high temperatures, and for most of the work a soil temperature maintained at about 24° C. in tanks was chosen. A typical result is given in table 2. In this experiment the inoculum was inserted beneath the surface of the thick phloem of roots about half an inch in diameter. The

control plants were wounded in the same manner as those inoculated. At the end of the experiment the plants invaded by the fungus to the center of the woody part of the root were usually so badly discolored that no microscopic examination was necessary to determine the extent of the fungus invasion. In other cases, the extent of fungus penetration was determined by staining microscopic preparations with the Gram stain.

TABLE 2.—*Infection in 3 roots of Melilotus alba, inoculated with cultures of Stagonospora meliloti from different host sources, after 40 days at 24° C.*

Culture No.	Host source	Number of plants			
		Uninfected	Phloem invasion		Wood invasion
			Slight	Extensive	
1287	*Melilotus alba*				3
1403	do			1	2
1260	*Medicago arabica*	2	1		
1347	*M. lupulina*	3			
1226	*M. sativa*	3			
1322	do	1		2	
1195	*Trifolium hybridum*				3
1232	do			3	
1184	*T. repens*	2			1
	Controls	6			

From the data given in table 2, supplemented with data from several similar trials, it appears that not all isolates from *Melilotus* are equally effective in infecting *Melilotus*. A similar situation was found with isolates from alfalfa, as shown in the results at Riverside given in table 1. While isolates from *Melilotus* as a group infect *Melilotus* more severely than any similar group yet obtained from the other host species, yet in the other host groups of isolates that have been made large enough to be significant some isolate has been found capable of infecting *Melilotus* to a degree approximating that of the less pathogenic isolates from *Melilotus* itself. Thus this method of inoculation has not served to disintuish any possible host-limited biological races in this species, though it has not been used sufficiently to lead safely to the conclusion that none exists. A thorough exploration of the extent to which and the conditions under which the fungus may pass from one host to another was considered to be beyond the province of this investigation.

MORPHOLOGY AND DEVELOPMENT OF THE FUNGUS ON HOST PLANTS

SUCCESSION OF THE THREE FRUITING FORMS ON HOST PLANTS

The fungus has been found producing on sweetclover three fruiting structures, of which the commonest and best known belongs in the genus *Stagonospora*. In addition to this, in the autumn a second fruiting form belonging in the genus *Phoma* develops on plants which are late in maturing or which continue producing flowers after the main crop of seed is set. The *Phoma* pycnidia appear around lesions in which the *Stagonospora* pycnidia developed earlier, usually near the bases of stems but sometimes upon small branches. This second or *Phoma* form appears to be distinct in character from the earlier or *Stagonospora*

form, and not a variation such as occurs in the *Stagonospora* on narcissus (*12*). The third form, belonging in the genus *Leptosphaeria*, appears on dead stems the following spring and continues to develop during the entire summer and autumn if these stems are well preserved.

The extent to which the three spore forms function in infection and in the perpetuation of the fungus is not clearly apparent from observation and studies. The *Phoma*-like spore form, found thus far only on second-year stems of *Melilotus* spp., does not appear to be suitably situated either in place or in time to serve as inoculum. In fact, no clear demonstration of its ability to infect has yet been made. The *Stagonospora* form, developing abundantly on dead first-year stems of sweetclover, is in excellent position to initiate the spring infection on that host. This may occur very early in the spring—it was found April 28 at Madison, Wis., in 1938—and at this time the pycnidia of this conidial form were almost always found on the overwintered stems standing in the midst of the young growth. The ascigerous stage was also found occasionally on these stems, but the ascospores were not mature. However, mature ascospores had been found a month earlier on first-year stems in a few locations; thus, if these served for inoculum they must have been wind-carried over some distance. That this may have been the case is indicated by the presence of the leaf spot on occasional isolated plants near which the fungus was not found on dead stems. After the middle of May the ascospores were found maturing abundantly. However, the infection of the upper foliage on rapidly growing plants rarely takes place unless these plants are spaced in rows. Thus the leaf spot, which seems from spring development capable of doing great damage to sweetclover, becomes less conspicuous during the period of rapid growth and may become abundant on the rosettes of leaves in late autumn.

On alfalfa no abundant early spring development of the leaf spot has been noted, even when dead stems of the previous year bearing pycnidia are present. In late spring the leaf spot has been found moderately abundant on plants isolated in thin stands or in rows, but not in thick stands. The ascigerous stage has been found sparingly with the conidial stage on occasional overwintered stems. Here, as with sweetclover, the relative abundance of the conidial stage can be taken as evidence that it serves chiefly as inoculum in the spring, while the ascigerous stage serves for widespread though sparse distribution of the fungus.

On the other host plants the fungus has not been observed as early in the spring as on sweetclover. Collections of the leaf spot have been made chiefly in the summer.

Early in the study of this fungus it became clear that not only was the chief specific character, spore size, highly variable, but that even the chief generic character, spore septation, was often absent not only in spores from cultures but also in those from the host. In 1935, herbarium collections were assembled and examined in the search for a species character of the fruiting structure as dependable as the staining reaction of the mycelium had proved in the identification of the fungus in infected roots. This character was found in the shape of the pycnidial neck or rostrum. When examined in paraffin sections of the fruiting structure, this rostrum was found to have a central canal narrower at the base than at the apex (fig. 3, *A*). The height of this rostrum varies in pycnidia in a single leaf spot, and when it is

short its flaring character is not prominent. However, pycnidia are usually abundant, and rarely need one search far to find a rostrum of the character illustrated. Pycnidia of other fungi on these hosts have been examined without a single rostrum of this characteristic form being found. Several of the descriptions of this fungus have mentioned the presence of a rostrum, but none has noted its peculiar shape, probably because this is readily observed only in well-prepared sections. Figure 3, *B*, illustrates a pycnidium of a closely related species, *Stagonospora recedens*, on *Trifolium pratense*, which is dis-

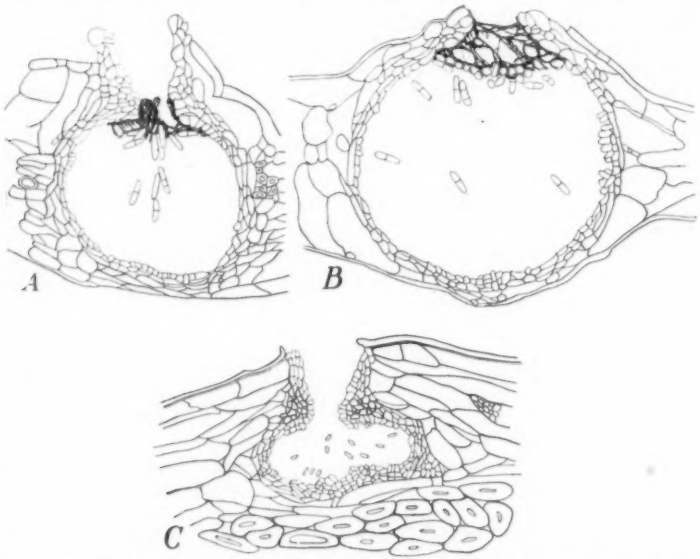

FIGURE 3.—Comparison of the two kinds of pycnidia of *Stagonospora meliloti* (Lasch) Petr. with that of *S. recedens* (C. Massalongo) n. comb.: *A*, A small pycnidium of *S. meliloti*, drawn from the Lucan collection for Fungi Columbiani No. 1335 and North American Fungi No. 2959; *B*, *S. recedens*, drawn from *Phleospora trifolii* Cav. var. *recedens* C. Massalongo in Krieger, Fungi Saxonici No. 1750; *C*, the *Phoma*-like stage of *S. meliloti*, drawn from a collection of the base of large stems of *Melilotus alba*. The thin parenchymatous rostrum with greater diameter at the apex, shown in *A*, is characteristic of *S. meliloti* but is absent from *S. recedens* as shown in *B*, though a short rostrum with isodiametric opening is sometimes found in this species. The characteristic rostrum occurs in the *Phoma*like stage of *S. meliloti*, as shown in *C*, when the pycnidia arise deeply in the tissue of large stems, but is often absent from pycnidia on small branches. × 230.

tinguished sharply from *S. meliloti* by the absence of the characteristic beak.

When the *Phoma* stage was found, the pycnidia were examined for the presence of this character. The pycnidium of this stage is developed in the parenchymatous exterior tissue of the stem, and where this is thin, on small branches, the neck of many of the pycnidia is so short that it has no noteworthy character. Wherever the origin of the pycnidium is deep enough to permit the development of the neck, it is like that of the pycnidia producing the larger spores (fig. 3, *C*).

SPORE MEASUREMENTS ON HOST PLANTS

Since the measurements of the *Stagonospora* type of spores in different collections were found to differ considerably, it appeared at first that these differences might be correlated with the host on which the fungus was found. To test this hypothesis spores were measured from seven collections from each of three of the principal hosts. The mean spore length of 50 spores taken at random was chosen as the datum most likely to show differences. It was found that on *Melilotus alba* the mean spore length ranged from 15.8μ to 19.3μ; on *Medicago sativa*, from 15μ to 19.5μ; on *Trifolium repens*, from 13.7μ to 17.7μ. These data show first of all a large variation in spore length among the collections on each host species. The spores of the fungus on *T. repens* appear shorter than those from the two other host species; yet if a single collection giving the lowest mean is excluded—a collection typical in all other respects—the range of measurements lies inside that of the measurement of spore length on alfalfa. In other words, the spore measurements on *T. repens* vary less than on the other hosts. Thus the fungus is not distinguished by consistent differences in spore size on these three hosts nor on any of the other hosts from which collections were examined later.

MORPHOLOGY AND DEVELOPMENT OF THE FUNGUS IN CULTURE

The general appearance of the fungus in culture has varied so much with age and origin that a simple precise description is impossible. When the isolate is derived from ascospores discharged upon malt agar the mycelial development from each group of spores is slow, with an early development of pycnidia. Conidia of both kinds planted in agar give cultures of much the same character. Pycnidia are formed at or near the center of growth. When this mycelial growth is transferred to agar slants, the transfer usually becomes a thick mat from which the mycelium spreads slowly from the central fungus mound. The aerial mycelium is scanty and white, yellowish, or gray in color. Isolates from mycelium in living tissue usually develop more rapidly on agar, with more mycelium and less fruiting, than cultures from spores. After several mycelial transfers the fungus may cease to fruit. The fruiting habit can usually be restored by plating macerated mycelium thickly in a thin layer of agar in capped bottles where evaporation is prevented. Potato agar with 1 percent each of lactose and dextrose, string bean agar, and malt agar are especially suitable for fruiting.

During the early part of the study of this fungus, isolates were obtained from the several host plants from as many localities as possible in the hope that on a uniform substratum the spores would show less variation in size than on the host plants and that possible host races of the fungus might be distinguished. This hope was completely disappointed. The mean length of a random sample of spores from one culture has been found as low as 13μ and from another as high as 18μ. While spores from some isolates appear to differ persistently from those of other isolates, these differences do not appear to be related to the host sources.

The morphology of the pycnidia of the *Stagonospora* stage in cultures shows remarkable diversity. This was especially true in one collection of 26 isolates from *Stagonospora* spores. When these isolates had been brought into fruiting habit and spores were plated in thin layers of agar, pycnidia were produced with little mycelium, with white mycelium, or in a black stromatic growth. In occasional cultures the pycnidial wall was very thick, like that mentioned and figured by Volkart (*46, fig. 4*) on *Trifolium repens*. In some cultures pycnidia had no ostioles or beaks; in others beaks were equal in length to the diameter of the pycnidium and opened at the surface of the agar or were oriented in all directions. Light was not found to stimulate the development of pycnidia or to influence their orientation.

The *Phoma* fruiting form of the fungus was not recognized in culture until after a collection of isolates from the *Phoma* stage and the ascigerous stage of the fungus had been obtained. It was first found in a group of cultures that had remained for some time by a north window in winter. Since it seemed likely that the low temperature had determined this development, poured plates were made from *Phoma* spores from three of the isolates and placed in incubators maintained at temperatures through the range within which the fungus had fruited freely in previous trials. After 2 weeks the plates held at 8° C. had the *Phoma* stage only; plates at 12° had only the *Phoma* stage in plates of one isolate, but both stages in plates of the other two isolates; at 16° the isolate having only the *Phoma* stage at the lower temperature now had both stages, while the other two isolates had only the *Stagonospora* stage; at 20° and 24° all cultures had only the *Stagonospora* stage. After some of the cultures had been removed from the 8° incubator and left at room temperature for 4 days, some of the pycnidia were found to have a distinct mass of *Stagonospora* spores at the bottom of the spore mass in the pycnidia, showing that with the change of temperature the pycnidia can respond with a change in the character of the spores produced. Not all pycnidia responded in this manner, nor were the two kinds of spores mixed.

Following this experiment, plates were poured from *Stagonospora* spores from old isolates—one from the fungus on sweetclover, three from white clover, and one from alsike clover—and these were placed in the same incubators. The isolate from sweetclover produced pycnidia with both spore forms at 8° C., and one of the isolates from *Trifolium repens* produced both forms at both 8° and 12°. Another isolate from white clover at 12° produced in some small pycnidia the first intergrading series of spores that has been found containing all intermediates possible between the two spore forms. The other isolates produced only the *Stagonospora* form at all temperatures. This limited experimental work serves only to indicate that low temperature is required for the development of the *Phoma* form, though all isolates did not respond alike. The presence of the two spore forms in the same pycnidium, as produced in these cultures, has not been found upon the host plant.

One point of difference in the method of discharge of the two kinds of spores from pycnidia deserves mention. The *Stagonospora* spores are discharged in threads or in slimy masses when water is present, while the *Phoma* conidia have not been seen to exude in masses in water but scatter singly. This difference in behavior seems to be associated with a characteristic difference in the development of the

contents of the pycnidium. The formation of the pycnidial cavity in the *Stagonospora* form is not precisely like that described by Dodge (*17*) in certain fungus parasites of fruits. The inception of the cavity is at or near the wall beneath the ostiole rather than at the center. This region is marked by the development of a small mass of material that stains brilliantly by the Gram method. Whether this material is the product of the disintegration of cells or is produced by cells is not clear. In culture several of these brightly staining spots may appear in the same pycnidium, and as many ostioles develop. Even on the host one pycnidium has been found having an ostiole on each surface. While there is no evidence to indicate that this staining material swells and presses against the pycnidial wall prior to spore discharge, it does form at the ostiole a plug which must absorb water from the exterior, and thus it may form the slime in which the extruded spores are at first embedded. On the other hand, this staining material has not been found in pycnidia of the *Phoma* form, and the spore mass in the pycnidium does not appear to swell appreciably when water is applied. Spores may be extruded by growth, especially in culture, but these spore masses do not appear to be at all coherent when water is applied.

TAXONOMY OF THE FUNGUS

SYNONYMY OF THE STAGONOSPORA FORM

The first and most widely known fruiting form of the fungus discussed here has been described and named repeatedly in consequence of its wide host range and variable spore length and septation. Other fungus species belonging in the genera to which this species has been assigned have been confused with it. Ascomycetous fungi found on occasion growing in association with it have been proposed as its overwintering form. Names thus applied have been brought together in ever-lengthening synonymies by mycologists, usually without recourse to cultures and often without examination of authentic specimens of all the species included. Thus the synonymies of this fungus include a group of fungi the relationship of which needs reexamination and restatement. The work reported here, together with some examination of the cultural behavior and the morphology of some of the fungi thus confused, appears to furnish the basis, not only for a new statement of the synonymy of this fungus, but also for the disposition of some of the names that have been incorrectly included.

Among the previously proposed synonymies, five have been selected which include all of the names proposed in all of them, except names derived by transfer to the unaccepted genera *Marssonina* and *Stagonosporopsis*—transfers that add nothing of interest and will not be mentioned further. The names in these synonymies, together with two others added by the authors, are grouped in table 3 opposite the names of the host plants (column 1) under which they were first described, or of the first-mentioned host plant in the list given in the original description. The citation number and date of each description follow each binomial. In the last column of table 3 the synonyms retained or proposed by the authors are designated. A brief statement of reasons for the inclusion or exclusion of each name given in a new description follows. To facilitate reference from this discussion to table 3 and for cross-reference within the table, original names applied with or without descriptions are numbered serially, while new combinations are given the numbers assigned the original names and

such repeated numbers are enclosed in brackets. Thus the names with bracketed numbers require no discussion. The complete synonymy is designated only in the last column of table 3.

TABLE 3.—*Summary of proposed synonymies of Stagonospora meliloti*

Host[1]	No.[2]	Name of fungus, citation No., and date	Synonymy[2] proposed by—					
			Davis (*14*)	Von Höhnel (*81*)	Horsfall (*24*)	Petrak (*32*)	Săvulescu and Sandu-ville (*41, p. 304*)	Jones and Weimer
Medicago arabica Huds. (=*M. maculata* Willd.).	1	*Ascochyta affinis* Jaap (*25, p. 35*), 1916						S
M. lupulina L.	2	*A. medicaginis* Bres. (*6, p. 326*), 1900		S				S
	3	*Marssonia medicaginis* Voss (*47, v. 4, p. 259*), 1892.				S		S
	[9]	*Septogloeum medicaginis* (Rob. and Desm.) Ell. and Ev.,[4] 1893.						S
	[3]	*Stagonospora medicaginis* (Voss) Bub. (*9*), 1916.				S		S
	4	*Diplodina medicaginis* Oud. (*36, p. 884*), 1903				S		
	5	*D. medicaginis* Oud. var. *phyllobia* Bub. (*9*), 1916.				S		S
	6	*Gloeosporium medicaginis* Ell. and Kell. (*20, p. 104*), 1887.		S				S
	7	*Pseudoplea briosiana* (Poll.) Höhn. (*22*), 1918				S		
M. sativa L.	8	*Septoria allantoidea* Berk. and Curt. (*4*), 1874		S				
	[8]	*Rhabdospora allantoidea* (Berk. and Curt.) Sacc. (*38, v. 3, p. 586*), 1884.						
	9	*Septoria medicaginis* Rob. and Desm. (*15*), 1847.		S		S	S	S
	[9]	*Stagonospora medicaginis* (Rob. and Desm.) Höhn. (*21*), 1910.		N			S	S
	10	*Ascochyta caulicola* Laub. (*27*), 1903					S	
	11	*A. lethalis* Ell. and Barth.,[5] 1903		S		S		
	[12]	*A. meliloti* (Trel.) Davis (*13*), 1919		S		S		
	12	*Gloeosporium meliloti* Trel. (*44*), 1883		S		S		
Melilotus alba Desr.	[12]	*Marssonia meliloti* (Trel.) Sacc. (*38, v. 3, p. 77*), 1884.				S		
	[13]	*Septoria meliloti* (Lasch) Sacc. (*39*), 1889				S	S	S
	13	*Sphaeria meliloti* Lasch,[6] 1842				S	S	S
	14	*Stagonospora carpathica* Bäumler (*3*), 1888				S	S	
	[13]	*S. meliloti* (Lasch) Petr. (*32*), 1919			N	N	N	N
Trifolium alpestre L.	15	*Septoria compta* Sacc. (*37, fig. 89*), 1877	S			S	S	S
	[15]	*Stagonospora compta* (Sacc.) Died. (*16*), 1912	S				S	S
	16	*Ascochyta confusa* Bub. (*8*), 1905	S					NN
	17	*A. trifolii* Bond. and Trus. (*45*), 1913	S		S			
	18	*Gloeosporium trifolii* Pk. (*31*), 1880	S					
	19	*Phleospora trifolii* Cav. var. *recedens* C. Massalongo (*29*), 1889.	S					
T. pratense L.	[19]	*Ascochyta trifolii* Siem. (*42*), 1914	S		S			
	20	*Pseudoplea trifolii* (Rostr.) Petr. (*32, v. 19, pp. 28–29*), 1921.				S		
	[21]	*Rhabdospora trifolii* (Ell.) Sacc. (*38, v. 3, p. 586*), 1884.						
	21	*Septoria trifolii* Ell. (*18*), 1882			S	S		
	22	*Ascochyta volkartii* Bub. (*28, p. 190*), 1910						NN
	23	*Phleospora trifolii* Cav. (*11*), 1889	S			S	S	S
	[23]	*Septoria trifolii* Cav. (*10*), 1888			S			S
T. repens L.	24	*Stagonospora dearnessii* Sacc. (*38, v. 10, p. 333*), 1892.	S			S	S	S
	25	*S. trifolii* Fautr. (*36*), 1890	S			S	S	S
	26	*S. trifolii* Ell. and Ev. (*19, p. 82*), 189				S	S	S

[1] Host plant first mentioned in original description.

[2] Serial numbers indicate original names proposed or given with descriptions; numbers in brackets indicate new combinations.

[3] N designates name chosen; S, synonym; NN, nomen nudum. The complete synonymy accepted by the authors is designated in the last column.

[4] ELLIS, J. B., and EVERHART, B. M. NORTH AMERICAN FUNGI [EXSICCATI]. Ser. 2, No. 2959. *Septogloeum medicaginis* (R & D) E & E. 1893.

[5] ELLIS, J. B., and EVERHART, B. M. FUNGI COLUMBIANI [EXSICCATI]. No. 1808. *Ascochyta lethalis* Ell. and Bart. 1903.

[6] KLOTZSCH, J. F. HERBARUM VIVUM MYCOLOGICUM [etc., EXSICCATI]. No. 370. *Sphaeria meliloti* Lasch. 1842.

NOTES ON SYNONYMS OF STAGONOSPORA MELILOTI (LASCH) PETR.

Since it appears that Petrak (*32*), in 1919, assigned the earliest specific name in the proper genus to the fungus considered here, his combination, *Stagonospora meliloti*, is the name that should be retained. Although type material has not been found for examination, no good reason for superseding the well-established name appears.

The following notes refer to synonyms of *Stagonospora meliloti* as given in table 3.

(1)[4] *Ascochyta affinis* Jaap.—In writing this description, Jaap appears not to have been aware that the fungus had previously been distributed by Vestergren[5] under the name *Marssonina medicaginis* (Voss) Magnus. Moreover he notes at the end of his description that *Septoria medicaginis* Rob. and Desm. may be the same fungus, which is indeed the case.

(2) *Ascochyta medicaginis* Bres.—Included from description and from examination of Dearness' collection.[6]

(3) *Marssonia medicaginis* Voss.—Bubák (*9*), who examined the original collection by Voss appears to show beyond doubt that this name belongs in the synonymy.

(5) *Diplodina medicaginis* Oud. var. *phyllobia* Bub.—The material from which this variety was described is stated to have been contributed by J. Lind for the "Fungi Imperfecti Exsiccati." However, it is not found in that collection either under the name assigned by Bubák or that given by Lind. Thus this fungus has not been available for examination, nor has the new varietal name been found cited elsewhere. The varietal description does not appear to define any character distinguishing the fungus either from the species to which it is subordinated or from that under consideration here. Therefore, the useless name is placed in this synonymy.

(6) *Gloeosporium medicaginis* Ell. and Kell.—Included after examination of the type and of a collection by Kellerman.[7]

(9) *Septoria medicaginis* Rob. and Desm.—Included after examination of the type.[8]

(15) *Septoria compta* Sacc.—Illustration and description leave no reasonable doubt of its identity.

(23) *Phleospora trifolii* Cav.—Included after examination of the type, kindly furnished to the writers by Dr. G. Pollacci.

(24) *Stagonospora dearnessii* Sacc.—The name given by Saccardo to *Stagonospora trifolii* Ell. and Ev. because the species name *trifolii* had been used previously in that genus. The description leaves no reasonable doubt of the identity of the fungus.

(25) *Stagonospora trifolii* Fautr.—The mention of the papillate pycnidia is good evidence of its identity, even if Allescher (*2*) had not mentioned its close resemblance to the two preceding species. No material has been found for examination.

(26) *Stagonospora trifolii* Ell. and Ev.—See (24) above.

[4] Numbers not italicized, used before fungus names, are the numbers used before these names in table 3.

[5] VESTERGREN, T. MICROMYCETES RARIORES SELECTI [EXSICCATI]. No. 1541. *Marssonina medicaginis* (Voss) Magnus. 1906.

[6] KABÁT, J. E., and BUBÁK, F. FUNGI IMPERFECTI [EXSICCATI]. No. 667. *Ascochyta medicaginis* Bres. 1911.

[7] ELLIS, J. B., and EVERHART, B. M. NORTH AMERICAN FUNGI [EXSICCATI]. Ser. 2, No. 2270. *Gloeosporium medicaginis* Ell. and Kell. 1889.

[8] DESMAZIÈRES, J. B. H. J. PLANTES CRYPTOGAMES DE FRANCE [EXSICCATI]. No. 1728. *Septoria medicaginis* Rob. 1848.

NOTES ON NAMES INCORRECTLY INCLUDED IN SYNONYMIES OF STAGONOSPORA MELILOTI

The disposal of names (table 3) that are not included properly in the synonymy is as follows:

(4) *Diplodina medicaginis* Oud.—Undoubtedly a synonym of *Ascochyta imperfecta* Pk. (*43, p. 712, footnote*).

(7) *Pseudoplea briosiana* (Poll.) Höhn.—See (20) below.

(8) *Septoria allantoidea* Berk. and Curt.—Could not be certainly determined by Brooks (*43, p. 713, footnote*) as identical with *Ascochyta imperfecta* Pk. upon examination of the type material, though the meager original description admits this possibility. A fungus identified as this has been distributed under the name *Rhabdospora allantoidea* (B. and C.) Sacc.[9] This fungus has spores up to 20μ in length and can hardly be *A. imperfecta*, nor has it characters which identify it as the fungus under consideration here.

(10) *Ascochyta caulicola* Laub.—In the course of the examination of *Ascochyta* on sweetclover, two species have been distinguished. First, a ubiquitous species that produces in nature and in culture an ascigerous stage identified as *Mycosphaerella lethalis* Stone. This is *Ascochyta lethalis* Ell. and Barth. The second species is less abundant and is not distinguished from the first by spore measurement or pycnidial character on the host plant. It is distinguished by cultural characters, by the hypertrophy of some of the host plants like that described by Laubert (*27*) as caused by *A. caulicola*, and by the absence of an ascigerous stage in culture and on the host so far as it has been observed at present. Under these circumstances, pending further investigation, *Ascochyta caulicola* Laub. is considered to be a valid name applying to the fungus that causes hypertrophy of the host plant, and not a synonym of *A. lethalis*.

(11) *Ascochyta lethalis* Ell. and Barth.—The imperfect stage of *Mycosphaerella lethalis* Stone, not often found fruiting abundantly on the living host. The ascigerous stage of this fungus has been obtained repeatedly in cultures on sweetclover stems stored at low temperatures. This fungus fruits rarely on leaves, which, when abundantly infected, soon shrivel and fall, thus behaving differently from leaves infected with *Stagonospora meliloti*. However, an excellent fruiting collection was made in the autumn on leaves of *Melilotus officinalis*, and another in the spring on *M. dentata*. The pycnidia on these leaves, like those on the stems, had no beaks.

(12) *Gloeosporium meliloti* Trel., or *Ascochyta meliloti* (Trel.) Davis.—Appears to have been placed correctly by Davis (*14*) as a synonym of *A. lethalis*, after examination of the original collection.

It is noted here that the fungus collected and distributed by Carlton under the synonym *Marssonia meliloti* Trel. is not that fungus, but is *Stagonospora meliloti*.

(14) *Stagonospora carpathica* Bäumler.—This name has been applied several times to the *Stagonospora* collected on *Melilotus* in the United States, in spite of the fact that conidiophores 8μ to 10μ long and 2μ in diameter are specified in the original description. No conidiophores have been seen in any specimen of *Stagonospora* examined.

[9] ELLIS, J. B., and EVERHART, B. M. FUNGI COLUMBIANI [EXSICCATI]. No. 1060. *Rhabdospora allantoidea* (B. and C.) Sacc. 1897.
——— and EVERHART, B. M. NORTH AMERICAN FUNGI [EXSICCATI]. Ser. 2, No. 3462. *Rhabdospora allantoidea* (B. and C.) Sacc. 1896.

Thus the name must be excluded from the synonymy. The writers have not seen any fungus on this plant to which the description applies.

(16) *Ascochyta confusa* Bub.—This name, suggested as a possible synonym, has been found without published description. The original collection has not been found. Therefore the binomial must be discarded as a nomen nudum.

(17) *Ascochyta trifolii* Bond. and Trus.—See *S. recedens* below.

(18) *Gloeosporium trifolii* Pk. See section on *Stagonospora recedens*, this page.

(19) *Phleospora trifolii* Cav. var. *recedens* C. Massalongo. See section on *Stagonospora recedens*, this page.

(20) *Pseudoplea trifolii* (Rostr.) Petr.—Too well known from recent studies to leave any room for suspicion that it has an imperfect fruiting stage.

(21) *Septoria trifolii* Ell.—Readily excluded from consideration by the size and shape of the spores as given in the description. Found upon dead leaves, it is probably a saprophyte.

(22) *Ascochyta volkartii* Bub.—This name, suggested as a possible synonym, has been found without published description. The original collection has not been found. Therefore the binomial must be discarded as a nomen nudum.

Stagonospora recedens (C. Massalongo) n. comb.

Three species (table 3, Nos. 17, 18, and 19) named as occurring on *Trifolium pratense* have been examined critically. The type collection of the oldest of these, *Gloeosporium trifolii* Pk., lent to the writers by the New York State Museum, has been found sterile. However, a fungus under this name corresponding well to the description and collected by Dearness [10] and several collections by J. J. Davis are deposited in the herbarium of the University of Wisconsin. These have been examined. Type material of *Ascochyta trifolii* Bond. and Trus. has been contributed by Dr. Bondartzeff. *Phleospora trifolii* Cav. var. *recedens* C. Massalongo has been seen in a collection by Krieger.[11] These collections appear to be of the same fungus, which is distinguished sharply from *Stagonospora meliloti* by the absence of the characteristic beak (fig. 3, *B*). Some of the pycnidia have short isodiametric papillae, as described by the authors, but they are not typical of those found in *S. meliloti*. In addition to this fungus, the collections by Davis and by Bondartzeff have the fungus that appears to have been incorrectly included with *Sporonema phacidioides* Desm. by Saccardo (*38, v. 25, p. 528*). Thus *S. meliloti* appears not to occur on red clover. The closely similar fungus on this host to which the three names given above have been applied appears to belong in the genus *Stagonospora*, especially in view of Korkhyrakoff's report (*26*) of spores with more than one septum. The species name applied by two authors to the fungus is not available, since it has been used for another species in that genus. Therefore, the varietal name applied by Massalongo is chosen for the species name of this fungus. Thus the name suggested is *Stagonospora recedens* (C. Massalongo) n. comb.

[10] Bartholomew, E. Fungi columbiani [exsiccati]. Cent. 46, No. 4538. *Gloeosporium trifolii* Pk. 1915.

[11] Krieger, W. Fungi saxonici [exsiccati]. No. 1072. *Phleospora trifolii* Cav. var. *recedens* C. Mass. 1895.

The description by Trusova (*45*) appears to be the most adequate when amended by the mention of the occurrence of spores with two or more septa.

TAXONOMY OF THE PHOMA FORM

The fruiting structure produced on stems of sweetclover in the autumn after the larger spored structures have ceased to develop belongs beyond doubt in the genus *Phoma*. Spores of this *Phoma* stage germinate in culture media and produce the *Stagonospora* stage in culture.

Two species in the genus *Phoma* have been described on sweetclover. One of these, *Phoma oleracea* Sacc. (*40, p. 549*), is certainly not the species at hand. Not only are the spore measurements given for the species too large, but in the type material lent to the writers by Dr. Dearness the pycnidia are found to be superficial, in striking contrast with the deeply situated pycnidia illustrated here (fig. 3, *C*.).

The description of the other species of *Phoma* on sweetclover—*Phoma meliloti* Allescher (*1*)—applies more closely to the material at hand. The minimum spore width of 0.5μ given in the description is too small for even the shrunken specimens in dry material, though these are very narrow indeed, and the pycnidia seem rather widely scattered on stems rather than gregarious as stated. The type collection of this fungus has not been seen; but a fungus distributed under this name by Krieger[12] has been examined. The collection was made in October, the right season of the year for the development of this form, and on small branches. A comparison of this material with a collection made near Madison on similar branches failed to show any differences whereby the pycnidia from the two collections could be distinguished. There were more pycnidia with characteristic flaring beaks on the Madison collection than on Krieger's collection, but a few were found on the latter. In both collections immature fruiting structures that may have been perithecia were found. Thus, on the basis of this comparison it appears that the name *Phoma meliloti* Allescher is correct as applied to the autumn fruiting structure of *Stagonospora meliloti*.

SYNONYMY OF THE ASCIGEROUS FORM, LEPTOSPHAERIA PRATENSIS SACC. AND BRIARD

The perfect stage of *Stagonospora meliloti* is well represented in European collections. It first appears in Rabenhorst,[13] under the name *Leptosphaeria dumetorum* Niessl, on *Melilotus alba*. The spores of the fungus in this collection, as measured by the authors, are 22μ to 26μ long, while a length of 18μ to 20μ is given in the description of the species. Rehm (*34*) distinguishes this collection as *Leptosphaeria dumetorum* Niessl f. *meliloti* Rehm, but gives the spore length as stated in the original species—presumably an oversight, though it is possible that Rabenhorst's collection was mixed, containing another of the several species of *Leptosphaeria* occurring on this host. Later, Berlese (*5, v. 1, pp. 55–56*) included Rehm's form in a new variety of another species, *L. eustoma* (Fries) Sacc. f. *major* Berlese, still giving the short spore measurement. While Berlese's figure (*5, table XLI*,

[12] KRIEGER, W. FUNGI SAXONICI [EXSICCATI]. No. 2284. *Phoma meliloti* Allescher. 1914.
[13] RABENHORST, L. FUNGI EUROPAEI [EXSICCATI]. No. 2238. *Leptosphaeria dumetorum* Niessl. 1877.

fig. 5) shows short, rather thick spores consistent with his measurement, still one of his three type collections,[14] in the packet that the authors have examined, contains typical material with spores more than 20μ in length. Thus this variety appears to be in part the perfect stage of *Stagonospora meliloti*.

It appears likely that the incorrect spore measurement stated by both Rehm and Berlese left the way open for the later descriptions of this species. The first correct designation of this fungus as a species appears to be *Leptosphaeria pratensis* Sacc. and Briard (*7*) described on *Medicago sativa*. The spore length is given as 28μ to 32μ. Berlese, who drew the fungus from the original collection (*5, v. 1, table LV, fig. 1*) gives the spore length as 28μ to 30μ. The authors have not seen this original material. Spores from the first collection made on alfalfa, discharged upon agar plates measured 25μ to 32μ in length, and thus were of the length stated in the original description. Spores from a collection made in the following year measured 21μ to 27μ in length, with a mean length of 23.6μ. From these data the opinion has been reached that the greater spore size stated for *Leptosphaeria pratensis* in the original description, while probably correct, is an extreme size in the species and is not a character distinguishing it from the *Leptosphaeria* previously discussed on *Melilotus alba*.

The fungus was later named *Leptosphaeria meliloti* by Hollós (*23*), who recognized (in a letter to the authors) the previous varietal designations stated above.

The collections of the fungus that have been verified by examination all being on *Melilotus* spp. except the American collection, which is stated to be on an undetermined shrubby plant, are listed with the proposed synonymy as follows:

Name:
Leptosphaeria pratensis Sacc. and Briard.
Synonyms:
L. dumetorum Niessl (in part in herbaria).
Rabenhorst, Fungi Europaei No. 2238, type.
Krieger, Fungi Saxonici No. 911.
De Thümen, Mycotheca Universalis No. 2248.
Petrak, Flora Bohemiae et Moraviae Exs. No. 2044.
Sydow, Flora Marchica (not numbered).
Vestergren, Micromycetes Rariores Selecti No. 726.
Ellis and Everhart, North American Fungi, 2d ser., No. 2614 (a).
L. dumetorum Niessl f. *meliloti* Rehm.
Sydow, Mycotheca Germanica No. 2528.
L. eustoma (Fries) Sacc. f. *major* Berlese (in part, as represented by Mycotheca Universalis No. 2248).
L. meliloti Hollós.

SUMMARY

A root rot of alfalfa, found first by the authors in California and in Wisconsin, is caused by a fungus previously known chiefly as a leaf-spotting fungus but having three fruiting forms, all of which have been described previously as independent species. This fungus is capable of producing a root rot of sweetclover also, though such root rot has been recognized but once in the field. The root rot progresses slowly, and is favored by high soil temperature. It is not readily distinguished macroscopically, but microscopically it is readily identified by staining

[14] De Thümen, F. Mycotheca Universalis (Exsiccati). No. 2248. *Leptosphaeria dumetorum* Niessl. 1884.

the fungus mycelium in roots or large stems with the Gram stain. Mycelium in leaves or in culture media is not thus stained. While the root rot develops best at a high soil temperature, the leaf spot develops abundantly at low temperature in early spring and late fall.

The chief imperfect stage of the fungus causing this root rot is defined here as a morphological species in the genus *Stagonospora*, identifiable on all of its host plants in three genera of legumes by the characteristically shaped rostrum, or beak, of the pycnidium. Cultures have been made of the fungus from nearly all of the host species known. While the fungus is variable in culture, none of the variations appear to be associated with the host origin. Some of the cultures from the more important host sources will infect sweetclover roots, though in varying degree. Cultures from sweetclover vary greatly in pathogenicity when inoculated into roots of that plant.

The first and most conspicuous fruiting form of the fungus appears to be properly designated *Stagonospora meliloti* (Lasch) Petr. This fungus has a large number of synonyms, which are discussed. The second form, found thus far only on *Melilotus alba*, in the autumn, has been previously described as *Phoma meliloti* Allescher. The ascigerous form, found thus far on alfalfa and sweetclover stems, in the spring, is *Leptosphaeria pratensis* Sacc. and Briard.

For a species of *Stagonospora* on *Trifolium pratense* closely resembling this species, the new combination *Stagonospora recedens* (C. Massalongo) is suggested.

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HISTOLOGY OF APPLE FRUIT TISSUE IN RELATION TO CRACKING[1]

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INTRODUCTION

Extensive loss often is incurred in the production of various fruits, notably sweet cherries (*Prunus avium* L.) and certain varieties of apple (*Malus sylvestris* Mill.), as a result of cracking of the skin and fleshy tissues sometime prior to harvest. Recent investigations have shown that in some of these fruits cracking is associated with abnormal acceleration of fruit growth or swelling as a result of a marked increase in water supply to the tissues. Verner and Blodgett (*12*)[2] found that cracking in sweet cherries was induced by an osmotic absorption of water through the fruit skin when this remained wet during prolonged periods of rain; and Verner (*10*) concluded that cracking in Stayman Winesap apples is promoted by increased water supply to the fruit tissues as a result of greatly depressed transpirational water loss under conditions of high humidity. In general, there seems to be a tendency for both cherries and apples to become increasingly susceptible to cracking as the fruits approach maturity.

Among different varieties of the same kind of fruit striking differences in tendency to crack have been observed. Bing, Lambert, and Napoleon sweet cherries are highly susceptible to this injury, while Republican, Eagle, and many other varieties are more or less free of it (*9*). Stayman Winesap apples sometimes crack severely, while apples of other varieties on nearby trees do not crack at all. Even among different individual fruits on the same tree remarkable differences in tendency to crack may be observed, some fruits cracking severely rather early in the season while others are still sound at maturity.

We may infer from such observations that some varieties, in general, and certain specific fruits of those varieties in particular, are in some manner peculiarly predisposed to cracking. Evidently those forces tending to promote unusually rapid swelling of the inner tissues through increased hydration are not alone responsible for this phenomenon. Some fruits, apparently, are able to accommodate these rapid rates of enlargement by corresponding growth or stretching of the peripheral tissues, while other fruits are not so adapted. The environmental influences that tend to promote cracking must, therefore, be regarded as merely contributory.

The present study was undertaken with the hope of finding a histological basis for the explanation of these diverse tendencies of different fruits to crack. Sections of fruit tissue of the highly susceptible Stayman Winesap apple were compared with similar tissues of several varieties in which cracking is rare. The latter varieties included Winesap, Winter Banana, Grimes Golden, Gano, and

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[2] Italic numbers in parentheses refer to Literature Cited, p. 823.

Arkansas. Especially careful examination was made of tissues adjacent to cracks in Stayman Winesap; and specimens of this variety that had cracked were compared with specimens which, although growing in the same orchard or on the same tree, had remained sound.

In a fruit such as the apple, which approximates a spherical shape, the most rapid growth in a tangential direction must occur in the outermost tissues. If growth or tangential stretching in these parts is unable to keep pace with growth or swelling of the underlying tissues (largely cortex in the apple), cracking may be expected to occur in the fruit periphery. The nature of tangential growth or stretching of the epidermal and hypodermal layers, roughly constituting the fruit "skin," is, therefore, of special interest in a study of cracking in this fruit. The relative positions of these tissues and the manner in which they are involved in cracking are shown by the photomicrograph in plate 1, *A*. Major consideration has been given to these tissues.

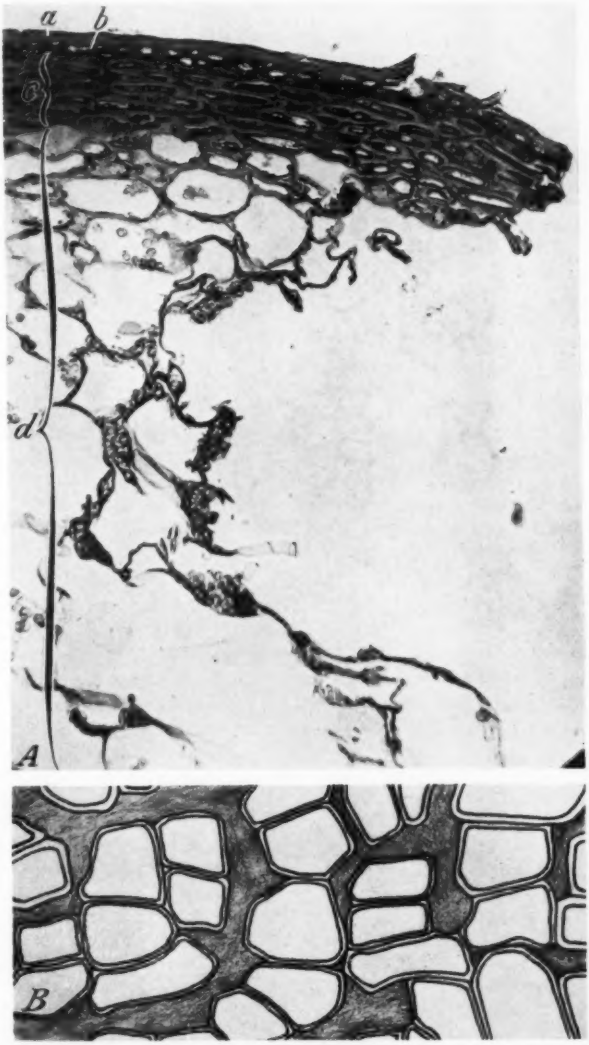
REVIEW OF LITERATURE

Since a review of literature on the subject of fruit cracking has been published recently (*10*), only such papers as have a direct bearing on the present study are considered here.

Tetley (*8*), in an anatomical study of Bramley Seedling apple, found that both cell division and cell growth continued later in the hypodermal layer of cells than in any other part of the cortex; and epidermal cells, she states, may continue to divide as long as the fruit is growing. As a rule, however, cell division in these regions decreases with advancing maturity, and much of the tangential stretching to which the peripheral tissues are subjected late in the season seems to be accommodated by a change in the direction of the long axes of cells in this region. In very early stages of development, when the apple is newly "set," the epidermal cells are somewhat brick-shaped, with their long axes lying in a radial plane; but, as growth proceeds, these cells seem gradually to yield to stretching until, at maturity, their long axes lie in a tangential plane. In the hypodermal cells also the tangential dimensions increase more radiply than the radial dimensions in the later growth stages. This change in shape of epidermal and hypodermal cells, as a result of tangential stretching, might conceivably provide for a considerable increase in surface area of the fruit without any appreciable cell growth having taken place.

Tetley states that the epidermis of Cox Orange Pippin, an English variety of apple that cracks readily, is characterized by deposition of cutin on the outer tangential, the radial, and the inner tangential walls of the epidermal cells. She considers that this type of cuticle offers less resistance to tangential stretching than a type of cuticle that covers only the outer tangential walls, or the outer tangential and radial walls of the epidermal cells. She seems to consider the type of cuticle of Cox Orange Pippin at least partly responsible for the tendency of this variety to crack.

Zschokke (*13*) observed that hairlike epidermal cells present in very young apples commonly are broken in the early growth stages of the fruit, leaving in the epidermis minute openings which subsequently become suberized and give rise to lenticels. Tetley (*8*), who also observed these hairlike structures, states that upon collapse of such turgid cells in a stretched epidermis small cracks in the skin may appear.



A, Transverse section showing one side of a shallow crack in a Stayman Winesap apple and the tissues involved in such cracking. × 125. *a*, Cuticle; *b*, epidermis; *c*, hypodermis; *d*, cortex. *B*, Tangential section through epidermis of Stayman Winesap apple showing cut-in (shaded portions) deposited between groups of epidermal cells. Traced from a photomicrograph. × 500.

According to Sorauer (*7*) cork formation usually occurs in the tissues subjacent to such small cracks, and this cork development may result in local russeting of the fruit skin. In subsequent growth of the fruit, cracks are likely to appear in such regions because of nonuniform swelling of the russet and the healthy areas.

Verner (*10*) considers that lenticel hypertrophy, which seems to be induced by the same environmental conditions that promote cracking, may sometimes constitute an initial stage in the development of cracks in Stayman Winesap apples. He points out that cracks in this variety occur most commonly in areas characterized by some abnormality of the peripheral tissues, such as russeting, lesions of apple scab (*Venturia inaequalis*), sunburn, or high skin coloration—conditions that seem to decrease the extensibility of these tissues.

Kertesz and Nebel (*3*), in a study of varietal differences in susceptibility to cracking in sweet cherries, found that those varieties that cracked most readily had smaller cells and thus, presumably, more cell-wall material than those resistant to cracking. When finely divided pulp of different varieties was placed on wet filter paper in funnels it was found that a relatively large percentage of the juice was retained by pulp of the varieties most susceptible to cracking, while in the noncracking sorts much of the juice passed through the filter. The greater retention of liquid by pulp of the varieties that crack badly was attributed to the imbibitional properties of the greater amount of colloidal substance in these fruits. These colloids of the cell walls, they conclude, probably exert a greater influence on absorption of water by the fruit in periods of rain than do the osmotically active constituents of the cell vacuoles; and they consider that varietal differences in colloid content serve to explain differences in their susceptibility to cracking.

METHODS OF PROCEDURE

The apple tissue samples used in this study were secured in 1933 and 1934 from nearly mature fruit grown at the Kearneysville Experiment Farm of the University of West Virginia. By means of a razor blade, wedge-shaped pieces of tissue were cut from the apple cheek in such a manner that each consisted of a 5-mm square of fruit skin and tapered on radial planes toward the core. In length these pieces of tissue ranged from 1 to 3 cm, the longer pieces usually traversing the cortex and including a portion of the pith of the apple.

As soon as a tissue sample was removed from an apple it was placed in a formalin-alcohol killing and fixing solution. The samples were prepared for embedding in paraffin by a routine procedure of dehydration in an alcohol series of increasing concentrations from 35 to 100 percent, and by clearing in a series of alcohol-xylol mixtures of increasing concentrations of xylol from 33⅓ to 100 percent. Paraffin with a melting point of 52° C. was used for embedding. Transverse sections 10 to 15 microns thick were cut with a rotary microtome, and these sections were stained with Delafield's haematoxylin and Sudan III according to the schedule recommended by Baker (*1*). The stained sections were mounted in glycerin jelly.

Photomicrographs were taken by means of a euscope equipped with a camera attachment. Length of exposure of the plates was controlled

through the use of a camera shutter attached to the substage of the microscope. A carbon-arc lamp served as a source of light.

Most of the sectioning, staining, and study of these tissues was carried on in 1935 and 1936 at the University of Idaho.

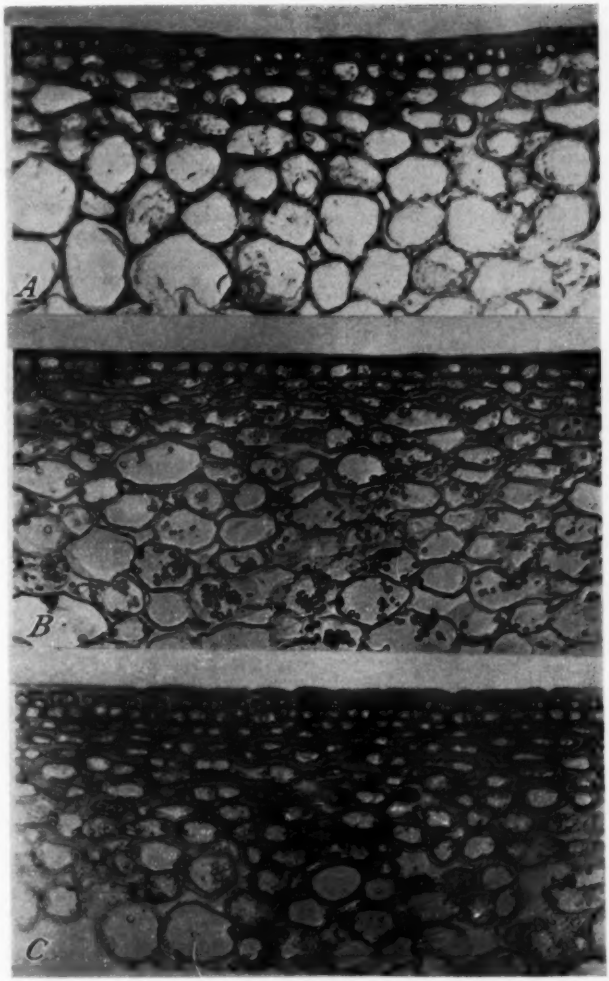
OBSERVATIONS

DIFFERENCES IN THE PERIPHERAL TISSUES OF CRACKING AND NONCRACKING APPLES

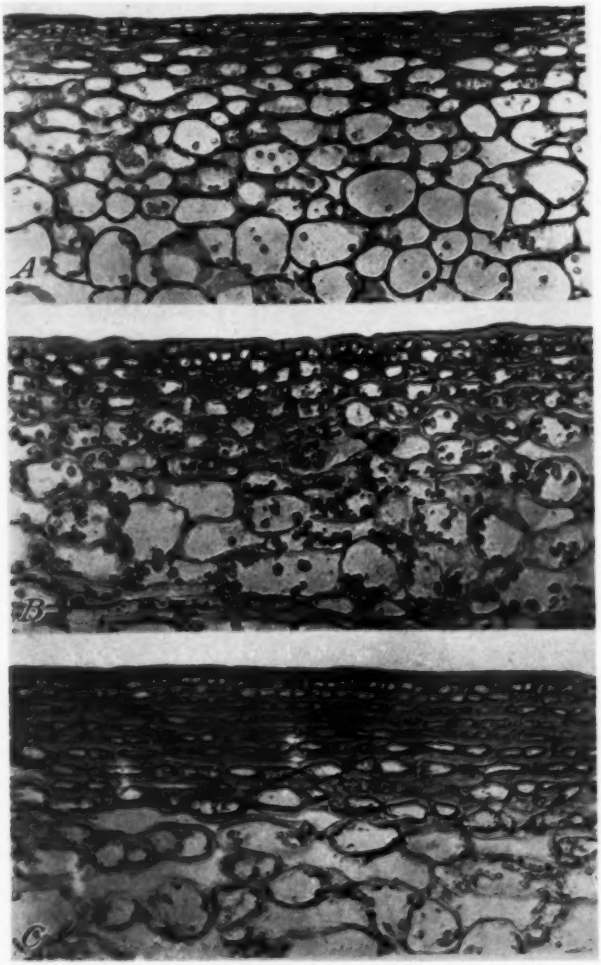
There were observed no consistent differences in structure of the cuticle or of the epidermis that might help to explain the tendency of some fruits to crack. In all varieties examined the cells of the epidermis had been pulled apart into small groups, as shown in plate 1, *B*, and the epidermis had remained intact only by virtue of increase in the cuticle, which had been projected into the interstices formed between the radial walls of cells that had been separated. In many cases cells of the epidermis were completely embedded in cutin and obviously were incapable of further growth. The development of this condition of the epidermis probably is attributable to premature cessation of division and growth in the cells of this layer, with a subsequent separation of these cells, one from another, as the epidermis is called upon to cover a larger and larger area of fruit surface. Preservation of the epidermal layer as a continuous, unbroken covering over the fruit is, therefore, dependent primarily upon continued deposition and stretching of the cutin that holds the epidermal layer intact. Since deposition of cutin may thus supplement deficient growth of the epidermis, the failure of the epidermal tissue itself to maintain an adequate growth rate cannot be regarded as contributing materially to the susceptibility of a variety to cracking. This view is supported by the fact that in varieties resistant to cracking, as well as in Stayman Winesap, the epidermis behaves in this manner.

In the hypodermal region of these apples striking differences were observed among the varieties studied, and these differences appear to be intimately related to the phenomenon of cracking. In Stayman Winesap the cells of the hypodermis, like those of the epidermis, showed unmistakable evidence of a deficient growth rate in the later stages of fruit enlargement; but this was not so of the varieties resistant to cracking. With few exceptions, the Stayman Winesap tissues examined were characterized by a more clearly defined hypodermal layer than was observed in any other of the varieties studied. In Stayman Winesap this layer consisted of much smaller and thicker-walled cells than were found in the subjacent part of the cortex, and these two regions usually were quite distinct. In most of the other varieties the hypodermal layer usually lacked a definite outline, the somewhat smaller cells of this region merging imperceptibly with the larger cortical cells. These different types of hypodermis are illustrated in plates 2 and 3, showing partial cross-sections of tissue from apparently normal fruits of Arkansas, Gano, Winesap, Grimes Golden, Winter Banana, and Stayman Winesap.

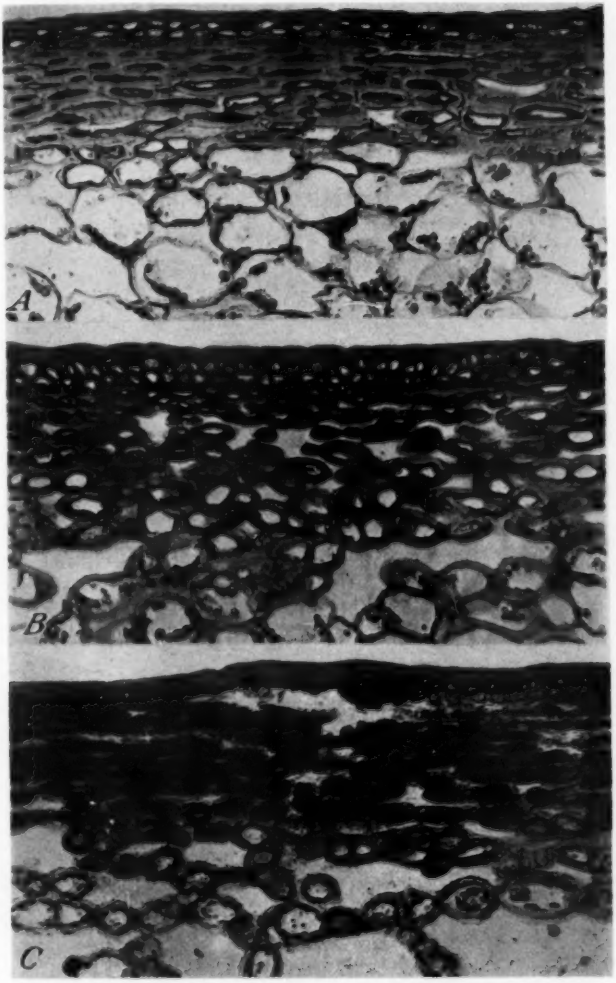
As shown in plate 3, *C*, tangential dimensions of the hypodermal cells of Stayman Winesap often are much greater than the radial dimensions, indicating that late growth of the fruit cortex was accommodated in the peripheral region principally by tangential stretching of the hypodermal cells rather than by cell growth. In the other



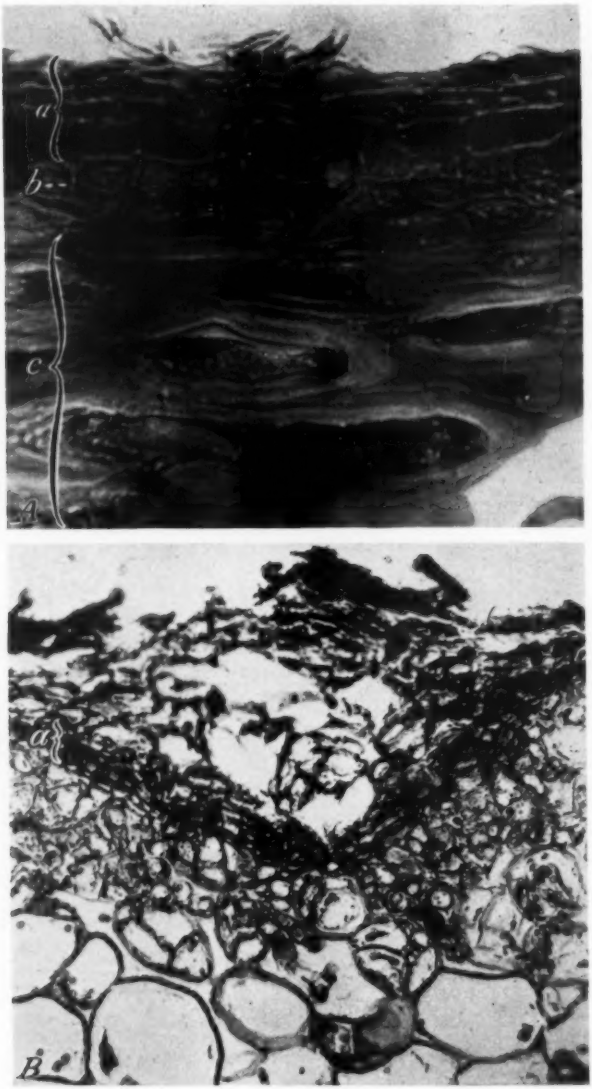
Transverse sections showing epidermal, hypodermal, and outermost cortical tissues of apple varieties resistant to cracking: *A*, Arkansas; *B*, Winter Banana; *C*, Winesap. × 125.



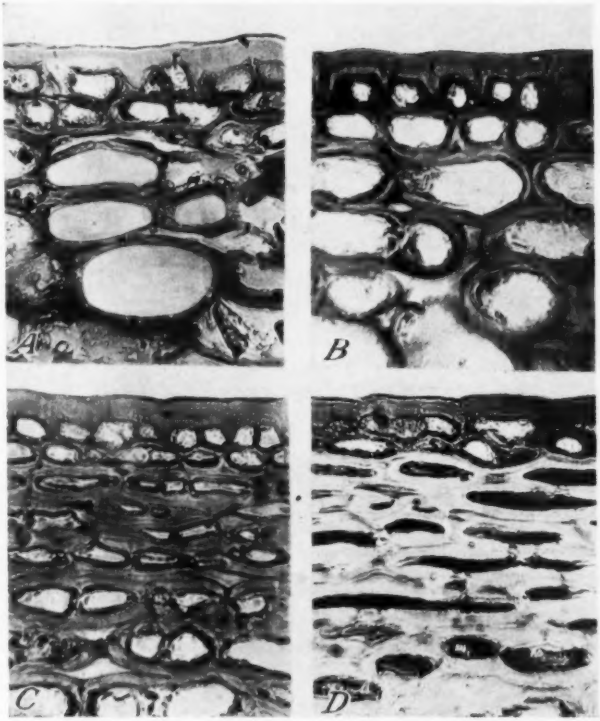
Transverse sections showing epidermal, hypodermal, and outermost cortical tissues of apple varieties: *A*, Gano (resistant to cracking); *B*, Grimes (resistant to cracking); *C*, Stayman Winesap (susceptible to cracking). × 125.



Transverse sections of Stayman Winesap apples showing modifications of normal peripheral tissues: *A*, adjacent to a crack; *B*, in a sunburned area; *C*, in an area probably injured by a lime-sulphur fungicide. × 125.



Transverse sections of Stayman Winesap apples showing: *A*, tangential elongation of hypodermal cells under cork tissue (*a*, cork; *b*, cork cambium; *c*, hypodermis). × 600. *B*, Hypodermal cells cut off from cortex by a deep layer of cork (darkly stained zone—*a*) and incipient cracking in the hypodermis. × 125.



Transverse sections of epidermal and hypodermal tissues: *A*, of densely shaded, noncracking type of Stayman Winesap; *B*, normal fruit of Arkansas, a noncracking variety; *C*, normally exposed but uncracked fruit of Stayman Winesap; *D*, adjacent to a crack in Stayman Winesap. × 300.

photomicrographs of plates 2 and 3, representing varieties resistant to cracking, cells in the hypodermal regions are nearly isodiametric in cross-sectional outline and we may assume that, in these varieties, the rapid expansion necessary in the outermost tissues during the later stages of fruit enlargement was taken care of by cell growth.

Sections of Stayman Winesap tissue adjacent to or including a crack (pls. 1 and 4, *A*) show even greater elongation of the hypodermal cells than was observed in normal tissue of this variety. In many of these cells the lumen is almost closed as a result of tangential stretching or a combination of stretching and radial compression from the swelling inner tissues. Cells such as these appear to have reached their limits of extensibility, and further growth of the fleshy portion of the fruit without commensurate growth in the hypodermis might be expected to sever these tissues and result in a crack. The more rounded hypodermal cells of noncracking varieties might, without further growth but simply by tangential elongation, permit a considerable increase in fruit volume without being torn apart.

TISSUE MODIFICATION IN SUNBURNED, SPRAY-INJURED, AND RUSSETED APPLES

Plate 4, *B* shows tissue of Stayman Winesap in a region characterized by sunburn, a condition of the fruit commonly associated with cracking. The loosely knit structure of the hypodermal layer suggests that growth of the cells in this region ceased or was greatly restricted before growth of the fruit as a whole came to an end, the hypodermal cells, consequently, having been pulled apart. A similar condition, the cause of which is not definitely known, is shown in plate 4, *C*. This apparent injury of the subepidermal layers is thought to be of the same origin as that described by Baker (*1*) and attributed by him to the use of a lime-sulphur fungicide late in the growing season. This condition, like that associated with sunburn, seems to predispose the affected region to cracking. We find here a disintegration of both epidermal and hypodermal tissues that would preclude further growth in these layers. Subsequent expansion in response to continued fruit growth should lead to cracking in an area thus affected.

Tissue modifications that appear in the condition commonly referred to as russeting, and which greatly increase the susceptibility of an apple to cracking, are shown in plate 5. Such russeting, or cork formation, takes place following injury to the cuticle. A layer of cork cambium assumes the position normally occupied by the epidermis, cutting off to the outside several, or many, tangential layers of cork cells, which constitute the scurflike russet. In the sections of russeted fruit that were examined there was little evidence of the formation of phelloderm cells that usually are cut off to the inside by the cork cambium. Beneath the cork tissue the hypodermal cells often were much elongated tangentially (pl. 5, *A*) as in nonrusseted tissues adjacent to or including a crack (pls. 1, 4, *A*, and 6, *D*).

In many sections through russeted areas it was observed that a narrow band of cork tissue extended into and, at some points, beneath the hypodermal layer (pl. 5, *A*, *B*). Hypodermal cells so separated from the cortex often presented a striated appearance owing to tangential stretching, and this tissue was badly disrupted. Apparently growth

had been terminated in this region while the fruit still was enlarging, in consequence of which the cells first were stretched to the limit of their extensibility and then were gradually pulled apart. This would add to the roughness of these russeted areas, and it might lead to incipient cracking as shown in plate 5, *A*.

TISSUE MODIFICATIONS IN DENSELY SHADED STAYMAN WINESAP FRUITS

In 3 years of observation, sound, densely shaded Stayman Winesap fruits growing in the innermost parts of the tree were never found to crack. Upon examination such shaded fruits seemed always to have thinner skins than specimens from more exposed parts of the tree. Apples selected at random in different exposures on several trees and enclosed in brown paper bags from 3 to 4 weeks before harvest seemed also to have abnormally thin skins when they reached maturity, and the incidence of cracking in these bagged apples was much less than in fruits normally exposed. In 1932 there were only 5.2 percent of cracked apples among 210 that had been covered, while among 190 that had been tagged and left exposed, 41.0 percent had cracked.

Zschokke (*13*) states that apples of a variety normally russeted, if shaded early in the growing season, remained free of russet and were thinner skinned than unshaded fruits. Magness and Diehl (*4*) found with several different varieties that the skin on the cheek exposed to the sun was thicker than the skin on the shaded cheek. As shown in plate 6, *A*, sections of tissue of Stayman Winesap apples from densely shaded parts of the tree lack the clearly differentiated hypodermal layer seen in sections of exposed fruits of this variety. The hypodermal cells of these shaded fruits resemble those of varieties that are not susceptible to cracking. They are rounded in outline, they are not clearly distinct from other cells of the cortex, and they lack the stretched appearance of corresponding cells in more exposed specimens of the same variety.

A and *B* of plate 6 show the similarity in structure of the hypodermal regions of a densely shaded Stayman Winesap apple and a normal fruit of Arkansas, a noncracking variety; and these may be contrasted with *C* and *D*, showing, respectively, hypodermal tissues characteristic of normally exposed and of cracked Stayman Winesap apples. The close relationship between degree of tangential stretching of the hypodermal cells and cracking is clearly evident in these illustrations.

Darkly stained cell inclusions, as seen in plate 6, *D*, were observed commonly in tissues adjacent to or including a crack. The nature of these inclusions has not been determined, but it is suggested that they might consist of dead protoplasm and other contents of cells that were killed sometime before the fruit was picked. Upon the death of these cells their growth would, of course, be terminated, with the result that the cells would be elongated and compressed as growth continued in the underlying tissues. Similar inclusions were present in cells of sunburned tissue, as shown in plate 4, *B*, in which the dark intracellular masses are to be seen adhering to the walls of the cells. These cells, largely separated from the subadjacent tissue through which their nourishment had been derived, must surely have died sometime prior to picking of the samples. Had they been elongated and flattened as were those shown in plate 6, *D*, they would have pre-

sented much the same appearance as these, with slitlike lumina filled by the dark inclusions.

Plate 6 also affords a comparison of cell-wall thickness in cracking and noncracking specimens of Stayman Winesap. The much greater thickness of hypodermal cell walls in plate 6, *D* than in 6, *A*, is characteristic of all of the cracked specimens examined except those in which spray injury or russeting were involved. Not only are the cell walls of cracked specimens thicker than those of densely shaded fruits but, as a rule, they are thicker than those of normally exposed but uncracked fruits of the same variety.

The cell walls of hypodermal tissue in normal fruits of Stayman Winesap are not appreciably thicker than in normal fruits of Winesap, which is a variety highly resistant to cracking. We cannot, therefore, regard the thickness of hypodermal cell walls in fruits of Stayman Winesap as an inherent characteristic that bears a causal relationship to cracking. We may, however, consider that the unusual thickness of these cell walls in specimens of Stayman Winesap in which cracking has occurred constitutes a special form of tissue modification to which this variety is especially prone, and which, perhaps, contributes to the tendency toward cracking in such specimens.

DISCUSSION AND CONCLUSIONS

It appears from these observations of tissue structure that the high degree of susceptibility to cracking in Stayman Winesap apples is attributable primarily to a tendency toward marked restriction of growth in the hypodermal layer late in the growing season while the fleshy portions of the fruit may be enlarging at a normal or an excessive rate. Under average conditions, when enlargement of the fruit is proceeding moderately, this restricted growth of the hypodermal cells is supplemented by tangential stretching, and a combination of these two processes (growth and stretching) enables the hypodermal layer to expand sufficiently to keep pace with enlargement of the main body of the fruit. At the same time, the epidermal layer remains intact through a gradual stretching, and increase in quantity, of the cuticle in which it is partially embedded. Thus, when growth proceeds at a moderate rate the fruit seldom cracks; but under conditions of very high humidity and its attendant low transpirational water loss, when tissue hydration is increased and the rate of fruit enlargement is abnormally rapid (*11*), the limit of extensibility of the hypodermal tissue soon is exceeded and cracking occurs. Growth measurements taken at times when Stayman Winesap apples were in the process of cracking have shown (*10*) that many noncracking specimens enlarge at more rapid rates than those that crack. The phenomenon of cracking cannot, therefore, be attributed to an exceptionally rapid rate of swelling in the cortex of cracking specimens, but must be regarded as a result of inadequate growth in their peripheral tissues.

Incipient cracking frequently involves only the cuticle and epidermis, the rupture of which gives rise to cork cambium that soon replaces the normal epidermal covering with less extensible cork tissue, or russet, which predisposes the affected area to more severe cracking at some later time. In deeper cracks the epidermal and hypodermal layers may occasionally behave independently as shown in plate 4, *B* and *C*, where the epidermis remained intact when cells of the hypo-

dermal layer were pulled apart; but it is evident that, as a rule, these two tissues remain closely knit and behave as one in such cracking. To some extent the incapacity to maintain an adequate growth rate in times of abnormal growth acceleration in the deeper tissues seems to include part of the cortex below the hypodermis, since cracks commonly involve the cortex to a depth of 10 or more cells. As previously pointed out, the configuration of an apple is such that expansion of its tissues in a tangential plane during growth must be greater in rate as the issue in question is farther removed from the central axis of the fruit. It is to be expected, therefore, that in times of unusual growth acceleration other regions of the cortex may, like the hypodermis, become involved in cracking by reason of inability to maintain a growth rate commensurate to that of the underlying tissue.

There is a rather common view that cracking indicates lack of strength or resistance on the part of the fruit "skin" to withstand the pressure imposed on it by internal growth. Thus, Tetley (*8*), in discussing cracking in Cox Orange Pippin apples, states that the "epidermis was unable to resist the rapid swelling of the cells within and had consequently cracked." In view of the observations here reported it is doubtful whether we should look upon these outermost tissues of the fruit as offering any considerable mechanical resistance to expansion of the deeper tissues. At best, the epidermal and hypodermal layers combined constitute a covering not exceeding one-fifth of a millimeter in thickness, and in some varieties especially resistant to cracking, such as Arkansas and Winter Banana, no clearly defined hypodermal layer can be distinguished. Cracking, therefore, should be looked upon, not as evidence of failure of these outer tissues to repress an excessive growth rate but rather as evidence of their failure to keep pace with it. In other words, the problem of cracking in the apple involves inability of the peripheral region to stretch or to grow as rapidly as it should when the fleshy portion of the fruit is enlarging at an abnormal rate.

There is, of course, a considerable strain on these outer tissues as growth takes place, and when this strain becomes excessive, as it does when there is inadequate tangential growth or stretching, we may expect these tissues to be torn apart first at their weakest points. It has been observed in many instances that cracks in apparently sound tissue had their origin in hypertrophied lenticels. In lenticel hypertrophy there is a considerable extension of cork tissue beyond the normal limits of the lenticel. According to Eames and MacDaniels (*2*), cork tissue in most plants is both inextensible and inelastic. In an epidermis under great strain because of disproportionate growth of epidermal and cortical tissues such localized areas of inextensible cork would mark points at which the tissues might first be pulled apart. This role of lenticels in cracking of apples probably is not of great importance, however, if we assume that the antecedent cause of cracking lies in failure of the peripheral tissues as a whole to maintain an adequate rate of growth. In the absence of lenticels cracks might occur almost as soon in uninterrupted tissue. No data are available to show the percentage of cracks that originate in hypertrophied lenticels, but observation has shown that cracks frequently appear without relation to them.

The high incidence of cracking in parts of the apple affected by russeting, sunburn, scab lesions, and other skin injuries can be attributed directly to these abnormalities. Such modifications of the fruit periphery involve considerable areas in which extensibility of the tissues is below normal. This is demonstrated by the photomicrographs of sunburned, spray-injured, and russeted tissues in plates 4, *B* and *C*, and 5, respectively. In the first two instances the capacity for explansion of most of the tissue comprising the fruit skin has been prematurely halted by death of many cells in this region. In the case of russeted areas the tendency to crack has been aggravated by partial disruption of the hypodermal layer or by substitution of inextensible cork for the normal epidermal and subepidermal tissues. Freezing-point depressions of the cortex underlying these modified skin areas have been shown (*10*) to be appreciably higher than under normal skin. Thus, abnormal tissue pressures in the cortex combine with subnormal extensibility of the peripheral layers of cells in making these sunburned, russeted, and otherwise injured portions of the apple especially prone to crack.

We can only speculate as to the probable causes of the structural peculiarities that render exposed fruits of a susceptible variety of apple more likely to crack than others. It is known that exposure of plant tissues to direct sunlight increases transpirational water loss. Fruits most exposed to sunlight also are most exposed to wind and general air movement, which would tend further to remove water from their tissues. Cell growth, which can take place only when the cell is turgid, might thus be checked in periods of intense sunshine and high evaporativity, owing to a water-saturation deficit of the tissues. According to Palladin (*5*) a severe water deficit promotes thickening of cell walls in the affected region. Thus, the premature cessation of cell growth and abnormal thickening of cell walls associated with cracking in exposed parts of Stayman Winesap apples might be considered to result from the drying effects of their immediate environment. Under conditions of prolonged hot, dry weather, especially when accompanied by soil-moisture deficiency, all of the fruits on a tree might be affected in a similar manner, but in various degrees, as a result of inadequate water supply to the outer layers of cells. Such a hypothesis would serve to explain the common observation that cracking is most likely to occur and affects the largest percentage of the crop if an abundant moisture supply is made available to the fruit following a protracted period of water shortage.

Cessation or restriction of growth in hypodermal tissues underlying russeted areas (pl. 5, *A*), might be due to a prolonged water deficit resulting from excessive moisture loss through the overlying zone of cork, which, according to Baker (*1*), is much more pervious to water than is normal cuticle. When all or part of the hypodermal region is separated from the cortex and from contact with vascular elements by a layer of cork, as in plate 5, *B*, further growth of the hypodermal cells might be inhibited by reason of restricted passage of nutrients and water through the suberin-impregnated zone below them. Occasionally both the epidermal and the subepidermal tissues are completely replaced by cork. In all of these conditions associated with russeting the normal peripheral tissues have been replaced by tissues that are much less extensible and that, therefore, increase the susceptibility of the affected area to cracking.

There is no evidence indicating that the theory of Kertesz and Nebel (*3*) regarding the role of colloids in cracking of cherries is applicable to this phenomenon in apples. In all of the varieties studied the smallest cells and the thickest cell walls (therefore, the greatest proportion of colloidal cell-wall materials) were found in the outermost tissues of the fruit. Upon excessive absorption of water in this region a condition should be approached just the reverse of that which causes cracking; that is, there should be expansion of the peripheral tissues in excess of the requirements imposed upon these tissues by growth of the deeper-lying cortex. In the cortex, where an abnormal rate of swelling by absorption of water seems responsible for the tissue strain that promotes cracking, the proportion of cell-wall material to osmotically active cell contents is much smaller than in the outer tissues, because of the larger cell sizes and thinner cell walls in the cortex.

Although some water may be absorbed through the skin of Stayman Winesap apples in periods of rain (*10*), most of the water supply permeating the fruit in times of cracking seems to enter through the usual channels of the vascular system. If the cell walls of the cortex were able to imbibe this water and swell appreciably before the peripheral tissues had been able to expand proportionately by the same process, we might expect cracking to occur in somewhat the manner suggested by Kertesz and Nebel (*3*) for cherries. However, there is no reason for supposing that the deeper cortical tissues would have access to an increased flow of water in the vascular system very long before it reached the hypodermal layer, where, as pointed out, colloidal absorption and swelling would tend to prevent rather than to promote cracking. It has been shown (*10*) that detached Stayman Winesap apples, with stems and calyxes sealed with paraffin, may crack severely when they are held for a period of 48 hours under water. Thus, even when an increased water supply is made available first to the small, thick-walled cells of the outer tissues by absorption directly through the skin, relatively greater swelling takes place in the region of the larger, thinner-walled cells of the cortex, and cracking results.

There seems to be little basis for assuming that the colloidal constituents of the cell walls would play a more important part in swelling of the fruit than do the osmotically active constituents of the cell vacuoles. As pointed out by Shull (*6*), cell solutes and the colloids of the cell wall are approximately in equilibrium with respect to the degree in which their water-saturation deficits are satisfied. Therefore the water-absorbing powers of the cell colloids should not appreciably exceed the osmotic value of the cell solutes at any given time. Although, as Kertesz and Nebel (*3*) point out, some colloids may exert an imbibitional force of as much as 2,000 atmospheres, such high values apply to colloids in a dry, or nearly dry state, whereas in the flesh of a fruit such as cherry or apple the actual colloidal pressure must be only a small fraction of its potential pressure due to the degree of hydration that exists in such succulent tissues. It is more likely that the excessive swelling of the fleshy portion of the fruit in times of abnormal water supply is due to the combined hydration forces of all those constituents of the cell and cell wall that tend to draw water to themselves, including both colloidal substances and cell solutes, which are component parts of the same equilibrated system.

SUMMARY

Different varieties of apple, and different individual fruits within a variety, exhibit striking differences in tendency to crack under the same environmental conditions. In an attempt to find a histological basis for the explanation of such differences fruit tissues of Stayman Winesap, a variety highly susceptible to cracking, and comparable tissues of five varieties usually immune, were sectioned and stained for microscopic study.

In most of the sections of Stayman Winesap that were examined, and especially in tissues in which cracking already had occurred and in those characterized by certain abnormalities commonly associated with cracking, there was definite evidence of premature cessation or retardation of growth in the peripheral tissues. In the epidermis the cells had been separated into small groups and the intervening spaces were filled with cutin, which thus preserved the continuity of the epidermal layer. This condition of the epidermis was evident in both cracking and noncracking varieties, however, and the structure and growth habit of the epidermis are not considered of great importance in determining the degree of susceptibility of a variety to cracking.

In Stayman Winesap the hypodermal layer of cells likewise showed clear evidence of an inadequate growth rate late in the season, as these cells were much elongated tangentially, suggesting that recent enlargement of the fleshy portions of the fruit had been accommodated in the hypodermis primarily by tangential stretching. In noncracking varieties the cells of the hypodermis were nearly isodiametric in cross-sectional outline, and evidently had maintained a growth rate proportional to that of the underlying tissues.

On the basis of these investigations there is advanced a hypothesis that the susceptibility of Stayman Winesap apples to cracking is due chiefly to premature cessation or restriction of growth in the hypodermal layer. Upon unusual acceleration of growth in the fruit cortex, as under conditious of very high atmospheric humidity with its attendant increase in tissue hydration, the limit of extensibility of the hypodermal layer soon is reached and further expansion of the tissues beneath leads to cracking. The phenomenon of cracking, therefore, probably should be regarded, not as a result of failure of the peripheral fruit tissues to repress an excessive growth rate of the cortex but rather as a result of their failure to keep pace with it.

This premature retardation of growth in the hypodermal layer seems to be related in some manner to exposure of the fruit to sun and general air movement, as the condition is greatly accentuated in tissue so exposed and is virtually absent in tissues of densely shaded fruits. Abnormal susceptibility to cracking in sunburned, spray-injured, and russeted areas is due to special types of structural weakness in the parts involved.

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PHYSIOLOGICAL CONDITIONS WHICH PRODUCE WING DEVELOPMENT IN THE PEA APHID[1]

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INTRODUCTION

An investigation was conducted during 1935 and 1936, from which data are presented that seem to clear up the existing confusion in regard to the appearance of wings among aphids, and to show more precisely the influence of the physiological "water balance" within aphids upon this phenomenon.

Various theories have been advanced to account for the presence or absence of wings among aphids, yet none of them has been entirely satisfactory. Production of wings has been associated with such epigenetic factors as temperature (*20*, *19*),[2] light (*16*, *24*), starvation (*9*, *10*), wilting of the host (*15*), crowding (*35*), and chemical composition of the host plant (*21*). The appearance of large numbers of alate aphids on wilted and crowded plants, either in early spring, midsummer, or under greenhouse conditions when the environments are different, suggests that climatic conditions are not the primary cause of wing development. The appearance of winged forms among progeny of aphids removed from plants and starved, indicates that unfavorable conditions of the host are only secondary influences on some physiological condition of the aphid. Recently it has been suggested by Ackerman (*1*) and Rivnay (*23*) that the physiological conditions which influence the wing formation are determined by the "water balance" within the aphid.

Correlation of moisture conditions and wing production are evident in the association of wilting of the host plant and starvation of its aphid population. However, the results of all investigators do not agree on this point. The first part of this investigation is devoted to experiments to show the probable reasons for these conflicting results, while the second part is devoted to a presentation of direct evidence relative to wing production, obtained from chemical, anatomical, and biological studies of the aphid.

METHODS

The pea aphid, *Macrosiphum pisi* (Kalt) was used in this investigation. Experiments designed to show the effect of aphid population upon appearance of winged forms were started with apterous fourth-stage individuals from parents which previously had fed on young succulent pea seedlings (*Pisum sativum* var. Alaska). These aphids were placed on experimental pea seedlings which were separated into the following height classes: 2 to 3 inches, 3 to 4 inches, and 5 to 6 inches. Three aphid populations obtained by placing 6, 12, and 20 adults on a plant, were tested on individual plants of the three

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[2] Italic numbers in parentheses refer to Literature Cited, p. 839.

height classes. These adults were allowed to reproduce on the plants up to 48 hours, at which time they were removed. Their progeny were allowed to remain on the plants until all had reached the third instar because this stage is the first in which the alate and apterous condition can be identified easily.

The pea seedlings used in the experiments were sprouted in sawdust and then transferred to Erlenmeyer flasks containing a complete nutrient solution. This solution contained 35.4 mg $Ca(NO_3)_2$, 20.4 mg KH_2PO_4, 18.5 mg $MgSO_4$. $7H_2O$, and a trace of $FeCl_3$. $6H_2O$ in 120 cc of distilled water.

The tops of these flasks were squeezed through 1-inch holes spaced 4 inches apart in strips of stiff linoleum 4 inches wide and 15 inches long. Care was taken to have the black surface of the linoleum upward and to have the holes small enough to hold the flask snugly by the rim. The linoleum was supported by two 15-inch laths placed under it against the necks of the flasks and at right angles to the supporting two by fours (fig. 1). Lantern globes placed on the flat

FIGURE 1.—Lantern-globe cages and working bench employed in the investigation.

surface of the linoleum made aphid-tight enclosures around the plants. Winged and wingless aphids, when shaken from the plants, fell onto the black surface of the linoleum where they were separated and quickly counted.

The starvation experiments were started with fourth-stage and adult apterous forms of apterous parentage. Some of them were caged on wilted plants and others were isolated in cheesecloth cages.

For quantitative analyses, large numbers of aphids were collected in alfalfa and pea fields by the sweeping method. Immediately after the aphids were collected, they were separated into the following

classes: (1) First- and second-stage nymphs; (2) fourth-stage apterous forms; (3) fourth-stage alate forms; (4) adult apterous forms; and (5) adult alate forms. Sorting of the aphids was best accomplished by dumping them onto the polished surface of a black photographic squeegee board. Aphids crawling out from the center of the board were separated and sucked into vials with the aid of a rubber tube. They were placed in air-tight rubber-stoppered vials in order that the moisture given up by the aphids in transit to the laboratory might be weighed and included in the determinations.

After each sweeping, a number of aphids in each stage were placed on balsam on a glass slide. A small amount of xylol and a cover glass were dropped over them, and the slides were taken to the laboratory where they were examined under the microscope. All aphids prepared in this manner and all unprepared specimens examined were identified as *Macrosiphum pisi*.

Collections from alfalfa (*Medicago sativa* L.) were made on May 7, 13, and 21 and those from peas on June 12 and 13, 1936. A sufficient number of stem mothers and their nymphs, apterous first-generation adults, and a large number of fourth-stage and adult alate individuals were collected on alfalfa. On peas a sufficient number of fourth-stage apterous forms was available, but only a very few alate adults. So, from both sources a sufficient number of the different forms was obtained for comparing the quantitative compositions.

Live weights of the aphids were obtained by subtracting the weights of the empty airtight rubber-stoppered vials at room temperature from the weights of the vials with aphids. Dry weights were obtained in a like manner after the vials had been 48 hours in a vacuum oven. A known dry weight of aphids was then placed in Whatman's No. 40 double acid-washed filter paper, put in an extraction thimble in a Caldwell extracting tube, and extracted with anhydrous ether in the usual manner.

The protein content of fat-free aphids was determined by the macro-Kjeldahl (*12*) and semimicro-Kjeldahl (*30*) methods.

Sugar analysis was determined by Elser's titration method (*7*). A known weight of 50 to 100 mg of fat-free aphids were weighed into a 50-cc beaker. About 4 cc of warm water was added and in this the aphids were macerated. The beaker with the aphid solution was kept for the next 12 hours between 35° and 40° F. and the total volume was then brought to 10 cc in a volumetric flask and 3-cc aliquots were examined in the usual manner for invert and higher sugars.

Serial sections of alate and apterous aphids were obtained by the paraffin method of preparation. Microdissections were made on aphids embedded in paraffin under water.

The volume of honeydew excreted by the pea aphid was measured by counting the number of drops expelled and obtaining their diameter under a stage micrometer. The drops were collected on sheets of cellophane which were placed daily inside the regular cages in which the aphids were confined.

MAXIMUM PRODUCTION OF ALATE PROGENY AND CONDITIONS WHICH INDUCE IT

Investigators generally agree that winged parents give birth to wingless progeny, (*33*, *25*), but all the results reported regarding the appearance of winged progeny of apterous parents do not agree. Kyber (*15*), Walker (*32*), Gentry (*8*), Mordwilko (*18*), and Davis and Whitehead (*6*) associated the appearance of winged forms with "underfeeding" of the aphids. Slingerland (*26*) reported that lack of food or crowding produced dwarfed apterous forms. Tannreuther (*29*) and Börner (*2*) associated the appearance of winged forms with abundance of food. Davidson (*4*) and Mason (*17*) found no increase of alate individuals on unsatisfactory food.

The probable explanation of these conflicting reports is partly shown by a simple experiment in which plants of three arbitrarily chosen heighth classes, were used each with three arbitrarily selected populations. The results of this experiment are given in table 1 and shown graphically in figure 2. These results indicate not only

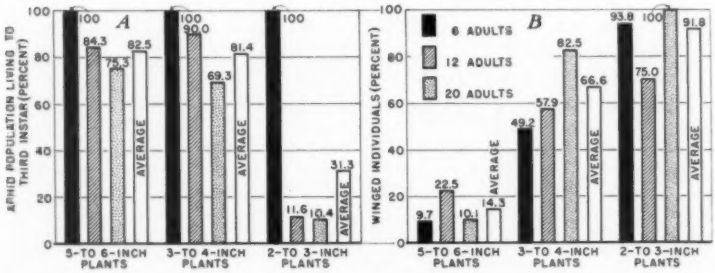

FIGURE 2.—Relation of ability of plants to support populations of aphids (*A*) and appearance of winged individuals (*B*).

that the ability of a pea plant to support an aphid population tends to decrease as the size of the plant decreases and as the size of the population increases, but also that the number of winged individuals tends to increase as the population increases, as the ability of the plant to support populations decreases, and as the size of the plant decreases. The correlation between appearance of winged forms and size of population is not so striking as the correlation between the appearance of winged forms and the size of plant. Tables 2 and 3 likewise show this lack of uniformity. However, these results may be explained by the possible variation in the physiological conditions of the plant or the aphids.

TABLE 1.—*The effect of size of population and of host upon the ability of the plant to support populations of pea aphids, and upon the appearance of winged forms*

Height of plants, and period adults were on them	Item	Data for populations produced by placing indicated number of adults on plants		
		6	12	20
5 to 6 inches; 48 hours	Adults—number	24	48	80
	Total progeny—do	321	631	935
	Average progeny per plant—do	80.2	157.8	233.8
	Progeny reaching third instar—do	321	532	704
	Average progeny per plant to 3d instar—do	80.2	133.0	176.0
	Progeny reaching third instar—percent	100.0	84.3	75.3
	Alate—number	31	120	71
	—do—percent	9.7	22.5	10.1
3 to 4 inches; 24 hours	Adults—number	36	72	120
	Total progeny—do	260	462	729
	Average progeny per plant—do	43.3	77.0	121.5
	Progeny reaching third instar—do	260	416	505
	Average progeny per plant to third instar—do	43.3	69.3	84.2
	Progeny reaching third instar—percent	100.0	90.0	69.3
	Alate—number	128	241	417
	—do—percent	49.2	57.9	82.5
2 to 3 inches; 20 hours	Adults—number	23	37	61
	Total progeny—do	160	275	268
	Average progeny per plant—do	40.0	68.8	67.0
	Progeny reaching third instar—do	160	32	28
	Average progeny per plant to third instar—do	40.0	8.0	7.0
	Progeny reaching third instar—percent	100	11.6	10.4
	Alate—number	150	24	28
	—do—percent	93.8	75.0	100.0

The foregoing experiment was modified to show the effect of previous nutrition of parent aphids on the production of alate offspring. Aphids were allowed to remain on test plants from 20 to 48 hours, and then transferred to a set of comparable plants which had not previously been infested. The results for the 2- to 3-inch plants and the 3- to 4-inch plants are given in tables 2 and 3. Aphids which fed on the 2- to 3-inch plants with one exception (table 2) and which had formerly given birth to large numbers of alate forms failed to maintain their previous ratio of alate offspring. Those aphids on the larger plants with one exception (table 3) increased their ratio of alate offspring.

These results support the contention of Kyber (*15*) that underfeeding is associated with the appearance of wings, provided the parent aphids have previously been on succulent food. They also support the contention of Slingerland (*26*) that underfeeding is associated with the appearance of "dwarfed" apterous forms, provided the parent aphids have previously been subjected to poor feeding conditions and have given birth to considerable numbers of winged forms. In one case an abundance of winged forms was obtained while in the other a larger number of apterous forms resulted.

TABLE 2.—*Effect of size of population on the ability of plants to support pea aphids and on the appearance of winged forms, compared on plants 2 to 3 inches in height during consecutive periods of 20, 24, and 36 hours*

Reproduction period of adults on plants	Item	Data for populations produced by placing indicated number of adults on 4 plants		
		6	12	20
First 20 hours	Adults.....number	23	37	61
	Total progeny.....do	160	275	268
	Progeny reaching third instar.....do	160	32	28
	Average progeny per adult.....do	7.0	.9	.5
	Average progeny per plant to third instar.....number	40.0	8.0	7.0
	Alate.....do	150	24	28
	.....do.....percent	93.8	75.0	100.0
Next 24 hours	Adults.....number	23	24	61
	Total progeny.....do	139	169	233
	Progeny reaching third instar.....do	139	133	122
	Average progeny per adult.....do	6.0	5.5	2.0
	Average progeny per plant.....do	34.8	33.2	39.5
	Alate.....do	129	107	114
	.....do.....percent	92.8	80.4	93.4
Next 36 hours	Adults.....number	18	24	37
	Total progeny.....do	172	145	313
	Progeny reaching third instar.....do	172	67	118
	Average progeny per adult.....do	9.5	2.8	3.2
	Average progeny per plant.....do	43.0	16.8	29.5
	Alate.....do	116	28	88
	.....do.....percent	67.4	41.8	74.6
Total	Adults.....number	64	85	159
	Total progeny.....do	471	589	814
	Progeny reaching third instar.....do	471	232	268
	Average progeny per adult.....do	7.4	2.7	1.7
	Average progeny per plant.....do	39.2	19.3	22.3
	Alate.....do	395	159	230
	.....do.....percent	83.9	68.5	85.8

TABLE 3.—*Effect of size of population on the ability of plants to support pea aphids and on the appearance of winged forms, compared on plants 5 to 6 inches in height during 4 consecutive 48-hour periods*

48-hour period adults were on plants	Item	Data for populations produced by placing indicated number of adults on 4 plants		
		6	12	20
First	Adults.....number	24	48	80
	Total progeny.....do	321	631	935
	Progeny reaching third instar.....do	321	532	704
	Average progeny per adult.....do	13.4	11.1	8.8
	Average progeny per plant.....do	80.2	133.0	176.0
	Alate.....do	31	120	71
	.....do.....percent	9.7	22.5	10.1
Second	Adults.....number	24	47	79
	Total progeny.....do	318	622	888
	Progeny reaching third instar.....do	318	618	786
	Average progeny per adult.....do	13.3	13.1	9.9
	Average progeny per plant.....do	79.5	154.5	196.5
	Alate.....do	1	206	260
	.....do.....percent	.3	33.3	33.1
Third	Adults.....number	23	[1] 36	79
	Total progeny.....do	260	442	771
	Progeny reaching third instar.....do	260	442	768
	Average progeny per adult.....do	12.6	12.3	9.7
	Average progeny per plant.....do	72.5	110.5	192.0
	Alate.....do	1	189	293
	.....do.....percent	.3	42.8	38.1
Fourth	Adults.....number	22	46	76
	Total progeny.....do	299	622	894
	Progeny reaching third instar.....do	299	622	894
	Average progeny per adult.....do	13.6	13.5	11.8
	Average progeny per plant.....do	74.8	155.5	223.
	Alate.....do	9	136	306
	.....do.....percent	3.0	21.8	34.2

[1] Some young of 11 adults on 1 plant escaped, therefore only data for 36 adults were included here.

RESULTS OF STARVING APTEROUS PARENTS IN THE FOURTH INSTAR AND IN THE ADULT STAGE ON SUBSEQUENT PRODUCTION OF ALATE PROGENY

Winged offspring appear among the progeny of starving apterous parents and of apterous parents placed on wilted plants, according to the results of Göldi (*9*), Gregory (*10*), Wadley (*31*), Ackerman (*1*), Reinhard (*22*), Shull (*25*), and others. This phenomenon also was noted in preliminary observations in this investigation. However, it was discovered that when fourth-stage aphids were starved or placed on wilted or semiwilted plants, few alate individuals appeared among the progeny (table 4).

TABLE 4.—*Difference in appearance of winged forms among the progeny of pea aphids starved in the fourth stage and of those starved in the adult stage*

[Wilted and semiwilted plants used]

Item	Fourth-stage aphids	Adult aphids
Adults.....number..	80	80
Progeny reaching third instar.....do....	153	247
Alate.....do....	5	81
Do.....percent..	3.3	32.8

This experiment was repeated with (1) aphids that were kept constantly on succulent pea seedlings as a check, (2) aphids that were removed from the succulent food for a period of 12 hours and then returned to their food plants, and (3) aphids that were removed for a period of 24 hours and then replaced on the plants. Aphids in group 1 were weighed when the experiment was started. All aphids of the latter two groups were weighed when they were removed and also when they were replaced. After they were returned to the plants they were allowed to reproduce for a period of 46 hours; then transferred to another group of plants and left for a period of 15 hours. Again they were transferred and left on a third group of plants for a period of 10 hours.

The results of this experiment are given in table 5. As in the previous experiment, the starved adults produced a higher proportion of alate progeny than those starved in the fourth stage. In addition, the experiment substantiates the view of Klodnitski (*13*) that the winged "anlage" are checked when prospective alate-producing adults are replaced on succulent growth.

TABLE 5.—*Difference in appearance of winged forms among the progeny of pea aphids starved in the fourth stage and of those starved in the adult stage*

Weight of aphids and period elapsed	Item	Fourth-stage aphids			Adult aphids		
		Not starved	Starved 12 hours	Starved 24 hours	Not starved	Starved 12 hours	Starved 24 hours
Average weight before starving...milligrams		1.71	1.70	1.70	2.44	2.29	2.41
Average weight before placing on plants..do		1.71	1.28	1.26	2.44	1.96	1.88
First 46 hours	Adults...number	10	8	6	10	8	7
	Progeny reaching third instar...number	37	8	3	117	49	42
	Alate...do	3	0	0	4	15	28
	...do...percent	8.1	0	0	3.4	30.6	66.7
Next 15 hours	Adults...number	10	4	5	10	5	5
	Progeny reaching third instar...number	60	16	30	85	43	29
	Alate...do	4	1	0	0	1	6
	...do...percent	6.7	6.3	0	0	2.3	20.7
Next 10 hours	Adults...number	6	3	4	10	5	5
	Progeny reaching third instar...number	31	15	26	56	30	23
	Alate...do	1	0	0	0	0	2
	...do...percent	3.2	0	0	0	0	8.7
Total	Adults...number	26	15	15	30	18	17
	Progency reaching third instar...number	128	39	59	258	122	94
	Alate...do	8	1	0	4	16	36
	...do...percent	6.2	2.6	0	1.6	13.1	38.3

QUANTITATIVE CHEMICAL ANALYSES OF ALATE AND APTEROUS APHIDS

Such phenomena as the lack of winged forms among the progeny of winged parents and of apterous parents subjected to unfavorable conditions in the fourth stage, and the inability to continue maximum production of winged forms, indicates that the controlling wing-producing mechanism is influenced by an accumulating and diminishing process of some sort. Ackerman (*1*) recognized the presence of such a changing process, and from a study of fat globules he concluded that the appearance of winged forms of the grain aphid was "dependent upon changes in the proportion or concentration of certain materials in the haemolymph as brought to pass by the rupture of the brown globules" (*1, p. 59*).

When the pea aphid was examined for type of globule content, there were found a large number of small, greenish, "plastidlike" structures that appeared to be fatty in nature, small clear globules and large clear globules, but no distinct brown globules.

Under close examination the number of globules, both the green and the clear, seemed to vary in the different aphids examined. This apparent difference prompted a quantitative analysis of different forms of the aphid. The results of the analysis are shown in tables 6 and 7. Differences in fat and moisture content are greater than differences in protein and sugar content. The fat content of the winged forms is greater than that of the apterous forms and the moisture content is smaller. Nymphs have a greater fat content than their mothers, but there is little difference in the moisture content of the two forms.

TABLE 6.—*Moisture, fat, and protein content of aphids collected from alfalfa and from peas in 1936*

Plant and stage of aphid	Moisture determinations				Ether-extract determinations				Protein determinations			
	Date collected	Live weight	Dry weight	Moisture content	Dry weight	Weight of ether extract	Fat content		Weight of fat-free residue	Weight of protein in fat-free residue	Protein content	
							Dry-weight basis	Live-weight basis			Dry-weight basis	Live-weight basis
		Milligrams	*Milligrams*	*Percent*	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Milligrams*	*Milligrams*	*Percent*	*Percent*
Alfalfa:												
Stem mothers	May 7	714.0	145.9	79.6	145.9	15.4	10.6	2.2				
	do	788.6	154.8	80.4	154.8	17.3	11.2	2.2	137.5	80.7	52.1	10.2
Nymph of stem mothers	do	367.6	78.7	78.6	78.6	10.6	13.5	2.9				
	do	433.2	93.5	78.4	93.5	13.1	14.0	3.0	80.6	34.6	36.9	8.0
Alate fourth instar	May 13	705.8	187.1	73.5	90.5	23.5	25.9	6.9	63.1	30.9	36.2	9.6
	May 21	324.0	84.5	73.9	226.3	57.7	25.5	6.7	109.1	53.5	36.5	9.6
Alate adult	May 13	565.7	148.0	73.8	113.6	29.0	25.5	6.7	80.2	44.5	41.3	10.8
	May 21	138.0	36.4	73.6	122.6	32.1	26.2	6.9	57.1	33.6	43.5	11.5
Apterous adult	May 13	927.6	207.7	77.6	96.8	14.6	15.1	3.4	156.4	86.6	47.0	10.5
	May 21	151.4	33.5	77.9	131.5	20.9	15.9	3.5	69.9	36.7	44.2	9.8
Peas:												
Nymphs	June 12	369.5	81.9	77.8	233.5	52.6	22.5	5.0	27.3	17.2	48.8	10.8
	June 13	197.7	42.8	78.3	180.6	43.3	23.9	5.2	27.5	17.3	47.8	10.4
Alate fourth instar	June 12	210.7	55.6	73.6	256.0	90.0	35.1	9.3	29.6	17.9	39.3	10.4
	June 13	159.6	42.1	73.6	162.7	54.5	33.5	8.8	29.2	17.3	39.4	10.4
Apterous fourth instar	June 12	251.9	56.7	77.5	220.0	52.4	23.8	5.4	29.2	16.3	42.5	9.6
	June 13	282.0	61.4	78.2	196.4	43.4	22.1	4.8	27.7	16.5	46.4	10.1
Apterous adult	June 12	489.7	111.1	77.3	388.0	81.7	21.1	4.8	30.5	18.4	47.6	10.8
	June 13	371.4	81.8	78.0	307.1	63.6	20.7	4.6	29.1	18.6	50.7	11.2

TABLE 7.—*Sugar content of aphids collected from alfalfa and peas in 1936*

Stage of aphid	Alfalfa							Peas						
	Date collected	Weight of fat-free sample	Invert sugar		Dextrin		Total sugar on live-weight basis	Date collected	Weight of fat-free sample	Invert sugar		Dextrin		Total sugar on live-weight basis
			Weight	On live-weight basis	Weight	On live-weight basis				Weight	On live-weight basis	Weight	On live-weight basis	
		Milligrams	*Milligrams*	*Percent*	*Milligrams*	*Percent*	*Percent*		*Milligrams*	*Milligrams*	*Percent*	*Milligrams*	*Percent*	*Percent*
Nymphs	May 13	7.4	5.5	0.8	7.6	1.0	1.8	June 12	36.2	1.9	0.9	1.6	0.8	1.7
	May 21	56.6	3.6	1.2	1.7	.6	1.8	June 13	79.3	3.5	.8	5.1	1.1	1.8
Alate fourths	do	61.5	1.3	1.4	2.7	.8	2.2	June 12	60.4	2.5	.8	3.1	.9	1.6
	May 13	62.9	3.6	1.1	2.3	.8	1.9	June 13	56.4	2.5	.8	2.5	.8	1.6
Alate adult	May 21	44.5	2.8	1.1	2.8	1.1	2.2	do	43.0	2.7	1.1	2.3	.7	1.7
Apterous fourth instar														
	May 13	77.6	6.2	1.5	4.4	1.1	2.6	June 12	81.2	3.1	.6	4.8	1.0	1.7
	May 21	64.4	5.6	1.5	3.7	1.0	2.6	June 13	76.3	2.9	.6	4.6	1.1	1.7
Apterous adult	do	119.5	9.2	1.3	5.5	.8	2.1	June 12	106.5	3.5	.6	5.4	.9	1.5
								June 13	106.8	2.7	.5	3.8	.6	1.1
								do	93.5	2.9	.5	4.1	.8	1.4

The protein content of adults is greater than that of immature forms and the protein content of alate forms is greater than that of apterous forms (table 6).

The sugar content of aphids collected from peas is noticeably lower than that of aphids collected from alfalfa. Apterous adults collected from alfalfa have a greater sugar content than all other forms collected on alfalfa, but apterous adults collected from peas have a lower sugar content than all other forms collected from peas.

The greater fat content of alate forms probably constitutes a source of reserve energy for use during migration. Nymphs have a greater fat content than their mothers probably because of the presence among them of a number that are destined to become alate. Apterous and alate adults might have a greater protein content than their nymphs because of the presence of developing young in their abdomens. Alate forms might have a greater protein content because of the presence of large wing-propelling muscles. Differences in the sugar content of aphids collected from alfalfa and those collected from peas are not well understood, but it may be that aphids feeding on peas take in more fluid than those feeding upon the less succulent alfalfa. As more fluid is ingested more will be egested and there will be a better opportunity to void the unnecessary sugars.

FOOD SOURCE, NATURE OF FOOD, AND STRUCTURE OF THE DIGESTIVE TRACT OF APHIDS

Aphids are known to penetrate to the phloem vessels (*5*, *11*, *27*, *28*). Davidson (*5*, *p. 51*) observed that—

> Aphids appear to feed almost continuously and large quantities of soluble carbohydrates are taken up in the cell sap. As these insects do not possess Malpighian tubules, the waste products, including surplus sugars, are passed out through the anus.

The Alimentary tract of the pea aphid was found to possess features similar to those of other aphids. Except for size and slight differences in arrangement, the digestive tract resembled that described for *Schizoneura lanigera* by Davidson (*3*). The entire length of the large intestine lies proximal to the stomach but does not lie partly enclosed in the stomach epithelium as was described by Knowlton (*14*) for *Longistigma caryae*. In some cases the stomach and large intestine were held together by tracheae, but these were easily pulled away. The cross sections show that the small intestine possesses a reduced lumen having the form of a T. The large cells of the thick intestinal wall contain large nuclei. The presence of these large nuclei indicate a probable excretory function. No malpighian tubules were found.

The passage of the excess fluid through the body of the aphid is summarized by Wigglesworth (*34*, *p. 45*) as follows:

> But instead of the superfluous fluid being taken into the blood and then eliminated by the Malpighian tubes in the manner just described [for blood-sucking forms], a dilated loop of the fore-gut, the "filter chamber," with very delicate walls, is invaginated into the rectum; the unwanted fluids are here absorbed, or perhaps simply filtered, directly into the terminal part of the hind-gut; and so come to be discharged again to the exterior as honey dew, manna, and such-like products.

Aphids must take in large quantities of plant sap in order to obtain the supply of nitrogenous food that is necessary for rapid reproduction. This sap is mostly moisture, and since a large amount of unnecessary carbohydrates are in solution, the problem becomes one of quickly voiding the liquid and the unnecessary waste products. The structure found in aphids apparently satisfies the requirements for rapid elimination of moisture and carbohydrates.

VOLUME OF HONEYDEW EXCRETED BY THE PEA APHID

Aphids appear to feed continuously, at least on succulent growth, so there is reason to believe that the quantity of honeydew excreted over a period of time is large. To find the exact amount would be a tedious procedure, but a relative amount was determined by counting the number of drops expelled by the aphid and measuring their diameter.

This was done with aphids from a crowded population and with those from an uncrowded population. Five groups of about 20 aphids each were taken from these populations and immediately placed on fresh succulent 8-inch plants. For a check, 23 aphids were taken from an uncrowded population and placed in a cage with no feed. The aphids from the uncrowded population (table 8) produced more honeydew than those taken from the crowded population, while aphids which were not allowed to feed on plants during the 24 hours produced no honeydew.

TABLE 8.—*Number and size of drops of honeydew excreted by pea aphids from a crowded population and by those from an uncrowded population*

Adults taken from—	Adults per plant	Drops of honeydew per 24-hour period			Adults taken from—	Adults per plant	Drops of honeydew per 24-hour period		
		Total	Average number per adult	Size			Total	Average number per adult	Size
	Number	*Number*	*Number*	*Microns*		*Number*	*Number*	*Number*	*Microns*
Uncrowded plants	20	190	9.5	600-800	Crowded plants	18	162	9.0	400-650
	20	176	8.8			19	115	6.1	
	16	166	10.4			17	146	8.6	
	18	203	11.3			15	116	7.7	
	16	157	9.8			15	125	8.3	
Total	90	892	9.9		Total	84	664	7.9	

The residues of the honeydews of the two groups of aphids appeared quite different under the microscope. This suggests that the composition varied, but as this information was obtained in only a preliminary way, no analyses were made. These observations, however, show that a careful study of this subject would add a great deal to the knowledge of the physiology of these insects.

After the completion of this investigation, a paper by Smith (*28*) appeared which describes a method of determining the relative rate of feeding of the mealy plum aphid by counting the drops of excrement thrown by the aphids onto sheets of paper. Smith found that aphids feeding as a group produced fewer drops of excrement per adult

than aphids which were isolated, and that under high temperatures fewer drops were produced than under lower temperatures.

The results of the two investigations are quite similar. Although the mealy plum aphid produced a greater number of drops per hour than the pea aphid, in both instances the aphids under the crowded condition produced fewer drops of honeydew than those under the uncrowded condition.

Since no honeydew was produced by the starved aphids, there appears to be a relationship between the volume of honeydew excreted and the intake of plant sap. The results obtained by Smith when aphids were subjected to high temperature, however, may be due not to the lack of intake of food but to the rapid evaporation of moisture from the bodies of the aphids. This investigation shows that rapid evaporation of moisture from aphids does take place, and that under low atmospheric humidity evaporation takes place more rapidly. Therefore it is not surprising that more alate forms should appear under these conditions (*23*).

ASSOCIATION OF WING APPEARANCE WITH A DECREASED VOLUME OF HONEYDEW AND A CONCENTRATION OF BODY CONTENTS

Crowding and starvation have been shown to be associated with an increase in the appearance of winged forms, and the volume of honeydew produced by aphids has been shown to decrease under such conditions. The appearance of winged forms, therefore, accompanies a decrease in the volume of honeydew excreted.

A consideration of the cause of the decreased amount of excretion brings us now to the realization that the concentration of the body content itself is associated with the appearance of wings. Evidence pointing to this was first presented in the starvation experiments, where it was shown that aphids lost weight very rapidly. After 12 hours, aphids previously weighing 2.29 mg had dropped to 1.96 mg. After 24 hours those weighing 2.41 mg had dropped to 1.88 mg (table 5). Later, in the trial moisture determination, this loss in weight was indicated as being due to loss of moisture. Aphids kept 3 to 4 hours showed a very definite decrease in moisture content as compared with those examined immediately after removal from the plants. On cloudy days this decrease was less noticeable than on bright, sunny days.

Since there were too few aphids in the starvation experiment to warrant quantitative determination, a large number of adults were collected from a pea field, mixed thoroughly in a screen cage, and then divided into three groups. Analysis of the first group was started 2 hours after collecting, that of the second group 30 hours after collecting, and that of the third group, 55 hours after collecting.

This experiment definitely showed (table 9) that as the moisture content decreased, the protein and ether extract increased. These results, which are to be expected, show more precisely the condition in which the evaporation of moisture leaves the aphid. They emphasize further that aphids not only excrete moisture in honeydew, but also give it up very rapidly by evaporation.

TABLE 9.—*Decrease in moisture content and weight, and consequent increase in protein and ether extract content of three groups of starved pea aphids*

Item	Results after—		
	2 hours	30 hours	55 hours
Weight of aphids.....grams	2.4530	1.2929	0.7291
Moisture content.....percent	72.9	65.3	50.4
Protein content.....do	12.5	19.1	30.0
Ether extract.....do	7.2	8.4	11.7

Winged appearance, therefore, is associated with concentration of body fluids brought about not only by the probable intake of more concentrated food but also by the rapid evaporation of moisture from the body of the aphid.

DISCUSSION

In the interpretation of these results, we must consider that the concentration of body fluids in the parent occurred either just before or within the period of the time of determination of the development of wings in the offspring. The length of this period is not definitely known (*25*), but whatever it is, the accumulation of the products or the concentration of body contents in the offspring must begin before this period is ended. If it occurs too early in the parent, for instance, when the prospective adults are still in the fourth stage, and if they are left on scant food, these products would be used before the adult had attained the reproductive stage, and, as was found to be the case, the progeny would have no wings. If parents are continued under unfavorable conditions, as was shown, the accumulated products would be used for the production of wings in the first young, but would not be sufficient to supply the later young. If adults are starved, as was shown, and then replaced on good succulent growth, the accumulated products would be used in the production of wings of the first young, but as the rate of the incoming fluid returned to normal and there was again enough to carry the waste products from the body of the parent, no more winged offspring would develop. Likewise, if prospective adults were kept on good succulent growth the products would not have an opportunity to accumulate and winged offspring would not develop. More winged offspring occurred on young plant seedlings, probably because the sap in these plants is more concentrated. Parents not ingesting a sufficient volume of fluid from these plants would soon have a large concentration of wing-producing products.

Winged adults subjected to unfavorable conditions would not be expected to produce many alate young because most of the products would be used in the development of their own muscles and wings. In nature they migrate to succulent growth and there the succulent food inhibits the development of wings. Only under the severest conditions would alate mothers be expected to accumulate enough of these products for the development of winged offspring.

Information presented in this investigation is applicable to circumstances wherein prediction of migrations is desired. Lack of available moisture or heavy early infestations are almost certain to

be followed by the appearance of large numbers of migrants regardless of other conditions. During the spring of 1936 a limited rainfall brought on such conditions and in one field under observation it was estimated that 90 percent of the aphids of the first generation were winged migrants. These early migrants cause an early initial infestation of field peas, which later leads to heavy losses where adequate control measures are not employed.

SUMMARY AND CONCLUSIONS

The appearance of winged offspring of the pea aphid is associated with a concentration of the body contents of the parent. The alimentary tract of the pea aphid is so constructed that it adequately eliminates waste substances only so long as fluid is ingested in sufficient volume. There appears to be no provision for voiding unnecessary products when the supply of fluid is insufficient for this purpose. Lack of fluid, together with rapid evaporation from the body of the parent aphid, causes a concentration of the waste substances. These accumulated "wastes" in the adult in the form of proteins and carbohydrates appear to initiate wing development. The appearance of apterous individuals appears to be due to (1) the lack of these "waste" substances in the parent, or (2) to the presence of a sufficient volume of fluid intake to carry them away in solution as honeydew.

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THE GROWTH CURVE OF SORGHUM[1]

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INTRODUCTION

Growers and investigators of sorghum (*Sorghum vulgare* Pers.) generally have observed the small size of seedlings and the relatively slow early growth of the plants, as compared with those of corn, even when grown under optimum conditions. Measurements of the growth of sorghum plants at some stages doubtless have been made previously, but curves showing the trend of this slow early growth have not been found in the literature. In 1930, N. V. Kanitkar, then soil physicist of the Poona Agricultural College, Poona, India, while visiting the United States, showed the junior writer an unpublished growth curve of sorghum based upon data he had obtained in India. This curve was of the unbalanced sigmoid type, having a long gradual slope during the first several weeks of the period, that depicted strikingly the slow early growth and rapid later growth of sorghums. It seemed desirable to the writers to investigate this problem in the United States and if possible to determine the cause of this unbalanced trend in growth rate. The experiments were conducted by the senior writer.

Sorghums are of tropical origin and usually grow slowly at the cool temperatures that frequently occur after relatively early planting in the Northern States. The sorghums in the experiments reported here, however, were planted in June and July under conditions of high temperature and irrigation in southern Arizona, which favored rapid germination and early growth. Thus unfavorable environmental conditions that might have retarded early growth were largely eliminated from consideration as a causal factor in the growth trend.

METHODS

The experiments were conducted at the University Farm, Tucson, Ariz., under irrigation, during the 4-year period 1931–34 and in 1936. Two grain sorghum varieties, Dwarf hegari and Double Dwarf Yellow milo, were planted on two dates in each of the first 4 years. The average date for the first planting was June 18 and for the second planting July 22. Soon after the plants were well up they were thinned to a single plant every 8 to 10 inches. The plants of both varieties produced tillers that developed about as well as the main stalks. Plants were harvested at 4-day intervals starting soon after the plants were up and ending when maturity was reached. The plants were cut as close to the ground as possible and a minimum of 10 plants was harvested at each sampling. The number of stalks (in-

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[2] The authors acknowledge the assistance of Charles Davis, assistant agronomist, Arizona Agricultural Experiment Station, in calculating the growth curves.

cluding tiller stalks) was counted in each sample, and all weights were computed to weight per stalk. The harvested plants were weighed at intervals until they had reached a constant air-dry weight. The heads, if any, were then removed and threshed, and weights of the heads and seed were obtained.

RESULTS

RATE OF GROWTH IN SORGHUMS

The increase in weight per stalk, including the heads, seeds, and leaves, of Dwarf hegari and Double Dwarf Yellow milo planted on two dates is shown in figure 1. The curves were calculated by the formula suggested by Robertson,[3] i. e., $\text{Log}\frac{x}{a-x}=K(t-t_1)$, where x equals the weight attained in time t; a is the total growth attained during the cycle; K is a constant, the magnitude of which determines the slope of the curve; and t_1 is the time at which the growth is one-half completed; or where x equals $1/2a$. It will be observed that the early growth of both varieties was very slow. This was especially true in the June 18 planting. It was not until 54 days after planting that a weight per stalk of over 10 g was obtained in Dwarf hegari. This period constitutes roughly one-half of the number of days between planting and maturity. The early growth of Double Dwarf Yellow milo was slightly greater than that of Dwarf hegari until about 58 days after planting. From then until maturity the weight per stalk of Dwarf hegari increased more rapidly than in the shorter variety Double Dwarf Yellow milo. The weight per stalk 54 days after planting represented 10 percent of the weight at maturity of Dwarf hegari and 14 percent of Double Dwarf Yellow milo.

A theoretical[4] symmetrical sigmoid (S-shaped) curve of growth, typical of many plant species, also is shown in figure 1. This curve is superimposed upon the weights of the Dwarf hegari planted on June 18. It will be observed that the curve of actual dry-weight increase of Dwarf hegari was below that of the theoretical curve at all stages of development until maturity was reached. At maturity, of course, the two curves coincide.

The growth curves of sorghum show a delayed but more abrupt upward trend, with the upper and lower ends asymmetrical in contrast to the symmetrical theoretical curve. The difference may be explained by the small size of the sorghum seedling relative to that of the mature plant, which necessitates the lapse of considerable time before the operation of the so-called "compound-interest" principle can produce large increases in weight.

When the two varieties were planted on July 22 there was a more rapid increase in early growth and a lower weight at maturity than when they were planted on June 18. This rapid early growth might be attributed to slightly higher temperatures at planting time in the July 22 planting. The mean temperatures at Tucson for June, July, and August from 1931 to 1934 were 79.5°, 87.6°, and 84.4° F., respectively. When the varieties were planted on June 18 maturity was reached the first week in October, but when planted on July 22

[3] ROBERTSON, T. BRAILSFORD. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 pp., illus. Philadelphia and London. 1923.

[4] RABER, ORAN. PRINCIPLES OF PLANT PHYSIOLOGY. 377 pp., illus. New York. 1928.

the varieties were not mature until the last week in October. The lower mean temperatures and shorter days during part of the growing period of the July plantings may have hastened heading and restricted

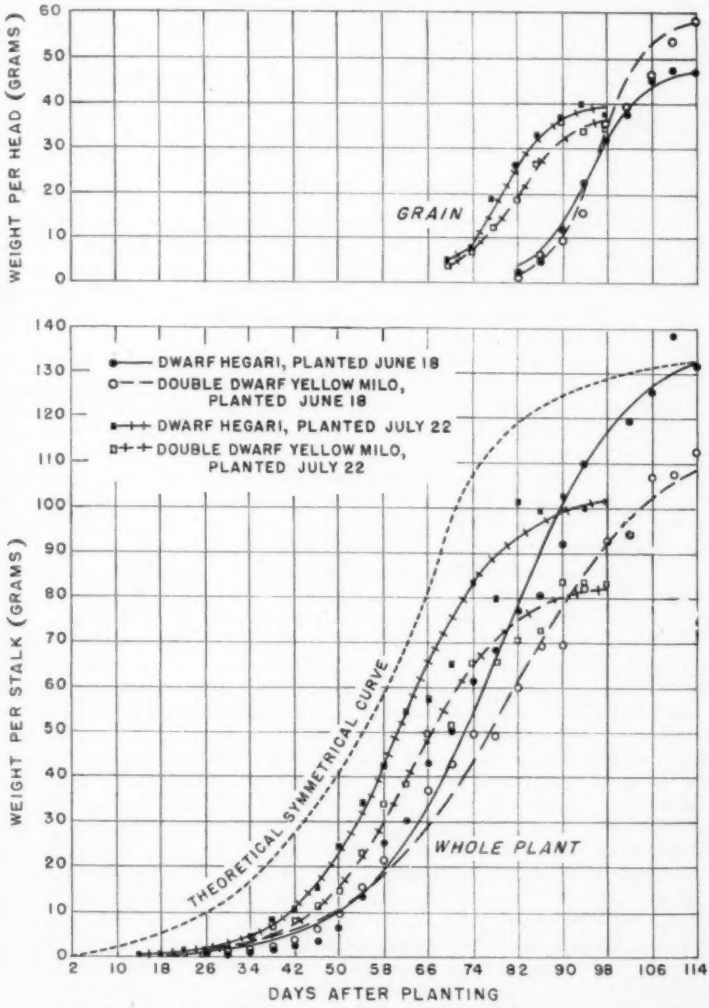
WEIGHT PER HEAD (GRAMS)
GRAIN
WEIGHT PER STALK (GRAMS)
DWARF HEGARI, PLANTED JUNE 18
DOUBLE DWARF YELLOW MILO, PLANTED JUNE 18
DWARF HEGARI, PLANTED JULY 22
DOUBLE DWARF YELLOW MILO, PLANTED JULY 22
THEORETICAL SYMMETRICAL CURVE
WHOLE PLANT
DAYS AFTER PLANTING

FIGURE 1.—Total air-dry weight per stalk and weight of grain per head of Dwarf hegari and Double Dwarf Yellow milo planted on two dates at Tucson, Ariz., 1931–34. The dots and circles represent the actual weights, and the lines the fitted curves.

vegetative growth, although the temperatures were sufficiently high during October to prevent delay in ripening.

The increase in seed weight per stalk of Dwarf hegari and Double Dwarf Yellow milo in the June 18 and July 22 plantings also is shown in figure 1. The poor fit in the curve for seed weight of Double Dwarf Yellow milo planted in June is due to damage by birds. However, both varieties yielded more in the June than in the July planting. In the June planting the seed yield of Double Dwarf Yellow milo was higher, but Dwarf hegari led in the July planting. The decreases as a result of later planting in weight per stalk and seed weight per head were 23 percent and 19 percent, respectively, in Dwarf hegari, and 26 percent and 41 percent in Double Dwarf Yellow milo. The Dwarf hegari evidently was better suited to late planting than Double Dwarf Yellow milo.

SEED SIZE AND EARLY GROWTH

In numerous experiments with sorghum seedlings, the junior writer has observed that varieties with large seeds produce larger seedlings and appear to grow more rapidly in the early stages than small-seeded varieties. It seemed that this apparent relationship between the size of seed and early plant growth might explain the growth trends just presented.

Mature stalks of some sorghum varieties often weigh nearly as much as mature cornstalks, although the seeds of grain sorghums are much smaller and usually weigh only about as much as average caryopses of wheat, oats, and barley; and seeds of some of the sorgos, particularly Sumac, weigh still less. Proso (*Panicum miliaceum* L.), a grain millet, has extremely small seeds for a cereal crop and makes a correspondingly slow early growth. The sorghums and proso in general have a wider ratio between seed size and final stalk weight at maturity than any other of the American cereal crop plants.

Plantings of cereal varieties having different seed sizes were made on July 13 and August 11, 1936. Included in this experiment were two varieties of corn (*Zea mays* L.), Surecropper and Krug; four varieties of sorghum (feterita, Double Dwarf Yellow milo, Dwarf hegari, and Sumac sorgo); and one variety of proso (Yellow Manitoba). The oven-dry weights per kernel, and per stalk 10 days after planting, of each variety are shown in table 1, and the stalk weights at intervals during periods up to 40 days after planting, which include only a part of the grand period of growth, are shown in figure 2. The stalk weights shown are averages from the two plantings.

In general, a close relationship was found between the size of seed and the size of seedling. The corn variety Surecropper had the largest seed and also the most rapid early growth, while Yellow Manitoba proso had the smallest seed and the least early growth. Krug corn made less early growth than the larger-seeded Surecropper variety but exceeded all of the sorghums. Among the sorghum varieties, feterita had the largest seeds and grew most rapidly during early stages, while Sumac sorgo had the smallest seeds and slowest apparent early growth. A distinct spread between sorghum varieties occurred from the sixteenth to the twentieth day after planting and the stalk weights then were in the same order as the seed weights, except that Dwarf hegari and Double Dwarf Yellow milo were reversed. The seeds of the latter variety usually are larger than those of Dwarf hegari but in these samples they were slightly smaller. The early growth of Double Dwarf Yellow milo exceeded that of Dwarf hegari, as shown in figure 1.

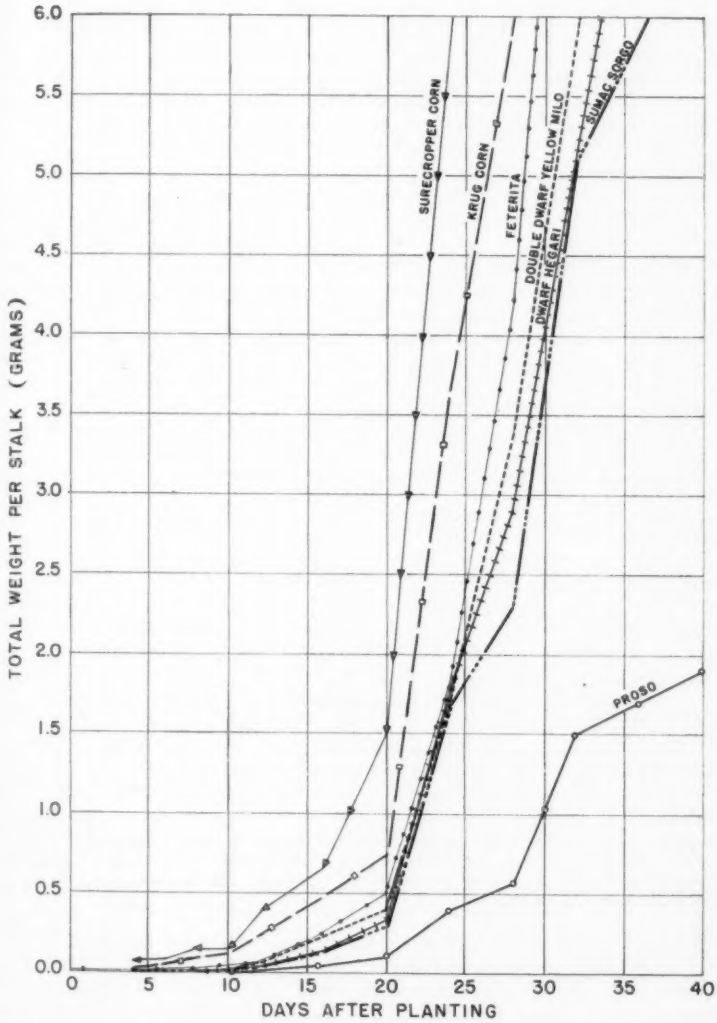

FIGURE 2.—Oven-dry weight per stalk of two varieties of corn, four of sorghum, and one of proso, at Tucson, Ariz., in 1936.

TABLE 1.—*Oven-dry weight per seed, and per stalk 10 days after planting and at different periods thereafter, of 7 cereal varieties*

Crop and variety	Weight per seed	Weight per stalk 10 days after planting	Period after planting for weight per stalk to equal weight per seed
	Mg	*Mg*	*Days*
Corn:			
Surecropper	407.8	149	14
Krug	188.0	112	11
Sorghum:			
Feterita	26.9	38	9
Dwarf hegari	25.9	33	9
Double Dwarf Yellow milo	23.4	37	8
Sumac sorgo	14.2	20	8
Proso:			
Yellow Manitoba	3.1	12	5

An inspection of figure 2 shows that after the seedlings had reached a weight of roughly 0.5g per stalk, the growth-curve rise for the next 5 days was strikingly and almost uniformly abrupt in all varieties. This is in conformity with the monomolecular autocatalytic law, upon which the usual growth curve is based,[5] in which increase in weight varies with the "active mass."

The weight per stalk 10 days after planting, which was previous to a pronounced upturn in the growth curve of any of the cereal varieties, is compared graphically with the weight per seed in figure 3, *A*. A

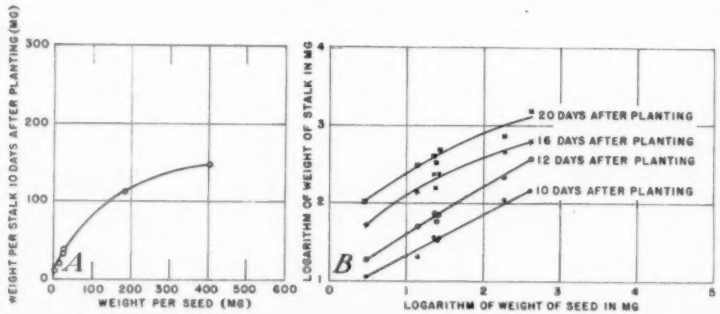

FIGURE 3.—*A*, Relation between weight per seed and weight per stalk 10 days after planting of corn, sorghum, and proso; *B*, relation between logarithms of the seed weights and of the stalk weights taken at various intervals up to 20 days after planting.

curvilinear relationship between stalk weight and seed size is indicated that closely approaches a logarithmic curve. When the logarithms of the two sets of weights are charted (fig. 3, *B*) the points fall approximately on a straight line. The relationship was linear also 12 days after planting. Later, 16 and 20 days after planting, when appreciable growth was occurring, the corresponding points in figure 3, *B*, form slight curves instead of straight lines, which indicates that factors other than seed size were operative in partly determining the weight per stalk at these stages. Ten and twelve days after

[5] ROBERTSON, T. BRAILSFORD. See footnote 3.

planting the logarithm of the weight of the seedling stalks thus varied directly with the logarithm of the weight of the seed of the corn, sorghum, and proso varieties included in the experiment. This is in conformity with the calculation of growth curves, which is based upon a logarithmic relationship between the weight of the plant (or "active mass") and the increase in growth. The results strongly suggest that the size of 10-day-old seedlings of the cereals investigated was determined by the mass of the seed and the apparent slow early growth of the sorghums and proso was merely a logarithmic function of seed size. A growth curve for barley obtained by Pope [6] showed a trend in early growth very similar to that of the sorghums shown in figure 1. Caryopses of barley have approximately the same average weight as those of the grain sorghums Dwarf hegari and Double Dwarf Yellow milo.

Ashby [7] has suggested that vigor in corn hybrids may be due in part to larger embryos, although this view has been challenged by Sprague [8] and others. Incidentally, Sprague's growth data, with one exception, show that the logarithms of seed and seedling weights are proportional.

SUMMARY

Sorghum seedlings are smaller and appear to be slower in early growth than those of corn. The increases in dry weight per stalk and grain weight per head of two grain sorghum varieties, Dwarf hegari and Double Dwarf Yellow milo, grown in the field under irrigation at Tucson, Ariz., from 1931 to 1934, are presented. A planting at a normal date, about June 18, and a later planting, about July 22, were made in each of the 4 years.

About 10 to 15 percent of the final dry weight of the stalks and heads was produced during the first half of the growing period. The growth curves of the two varieties in both plantings indicated a much slower increase in growth in early stages of development and a more rapid increase at later stages than would be expected from the usual symmetrical sigmoid growth curve typical of many plants.

Two varieties of corn, four of sorghum, and one of proso, representing a wide range of seed sizes, were planted in the field in 1936, and the rate of early growth was determined. A close relationship was found between the size of seed planted and the dry weight per stalk in the early stages of plant development. In young seedlings of corn, sorghum, and proso, 10 and 12 days after planting, the logarithm of the weight per stalk was directly proportional to the logarithm of weight per seed. Seedling size thus was a logarithmic function of the "active mass" in the seed. The wider ratio between the weight of the seed and the weight of the mature stalk appears to explain for the most part the smaller seedlings and apparent slower early growth of sorghum and proso, as compared with corn, under optimum growing conditions.

[6] POPE, MERRITT N. THE GROWTH CURVE IN BARLEY. Jour. Agr. Research 44: 323-341, illus. 1932.

[7] ASHBY, ERIC. STUDIES IN THE INHERITANCE OF PHYSIOLOGICAL CHARACTERS. 1. A PHYSIOLOGICAL INVESTIGATION OF THE NATURE OF HYBRID VIGOUR IN MAIZE. Ann. Bot. [London] 44: [457]-467, illus. 1930.

[8] SPRAGUE, G. F. HYBRID VIGOR AND GROWTH RATES IN A MAIZE CROSS AND ITS RECIPROCAL. Jour. Agr. Research. 53: 819-830, illus. 1936.

CORRELATIONS BETWEEN BIOLOGICAL ESSENTIALITY AND ATOMIC STRUCTURE OF THE CHEMICAL ELEMENTS [1]

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INTRODUCTION

An examination of the data on the mineral nutrition of organisms led to the discovery of close correlations between the list of the known essential elements and the quantitative data of atomic physics, permitting of tentative, if vague, deductions as to their total number and the identity of those as yet unknown. Though these correlations have been evident to the writer for some time, considerable hesitancy has been felt in calling attention to them in view of the importance of the questions involved and the possibility of error in interpretation of the data. The positive proof subsequently available of the essentiality of manganese and the discovery of the essentiality of molybdenum (*1*, *6*),[3] cobalt (*9*), and gallium (*7*), which one after another have fallen into their proper niches in the tables bringing out these relations, at length convinced the writer that these data should be made available for critical examination by other investigators. Substantiation would imply that the particular chemical elements required in the functioning of protoplasm are essential because of the inner physics of the atoms, and the quantum-mechanic relations of the atoms of these elements to one another.

REVIEW OF LITERATURE

Casual comments on the elements required by organisms are numerous and range from the assumption that organisms may require all 92 elements to one that some organisms have special requirements not shared by others. Attempts at correlations have been few and not very successful, owing to the limited knowledge hitherto available. Thatcher (*8*) proposed a classification of the chemical elements based on their biological properties, whereas Frey-Wyssling (*2*) considered that he had found a relation to exist between biological essentiality and position in the chemical periodic table. Lendle (*3*), on the other hand, confined himself to pointing out analogies in biological effects of homologous elements. Pirschle (*4*, *5*) has reported at length on the effects of homologous elements in *Aspergillus*.

RELATION OF BIOLOGICAL ESSENTIALITY TO ATOMIC STRUCTURE

A comparison of the essential [4] with the nonessential elements of the standard chemical periodic table with respect to position discloses evidence of considerable interest. This is brought out in table 1,

[1] Received for publication August 8, 1938; issued December, 1938.
[2] The writer acknowledges his indebtedness to Dr. F. L. Mohler, Chief of the Section of Atomic Physics, Bureau of Standards, U. S. Department of Commerce, for aid in the preparation of this manuscript.
[3] Italic numbers in parentheses refer to Literature Cited, p. 851.
[4] All the chemical elements definitely known to be essential, whether for animals or plants, are included. Limitations of space prevent the inclusion of full citations, nor are they nesessary in the case of the majority of the elements.

a reproduction of the standard chemical periodic table in condensed form, showing only the positions of the essential elements. Several facts stand out. Three, and no more than three, of the essential elements are found in each of five of the nine groups. Three contain two elements each. Each group containing three essential elements has one or two of them in the main group and not more than one in one or both of the subgroups. Groups 0 and VIII appear to form exceptions to these statements. Hydrogen, however, may be assumed to be a member of group 0, in which there are, of course, no subgroups. Group VIII contains two essential elements, but perhaps provision should be made for the addition of a third for reasons that will appear later. It may be noted that hydrogen and the two groups requiring special mention with respect to the property of essentiality are the same that have caused difficulty to the chemist in the classification of the chemical elements. On the basis of these facts, it seems probable that a total of three essential elements will also be found eventually in groups IV and V. The difficulties of table 1 are inherent in the chemical periodic table as a medium for bringing out these biological correlations.

TABLE 1.—*The biologically essential elements arranged in the form of the chemical periodic table*

Elements in the chemical periodic table in group [1]—

0	I		II		III		IV		V		VI		VII		VIII	
H	Na		Mg		B		C Si		N P		O S		Cl			
	K	Cu	Ca	Zn	(Sc)[2]	Ga	[3]*	*	*	*	Mo		Mn	I	Fe	Co

[1] Group 0 may be considered to contain no subgroups and group VIII no main group.

[2] Other evidence on hand renders quite probable the essentiality of scandium to *Aspergillus niger*, but will be presented at another time in connection with other matters if and when its essentiality to this organism is further verified.

[3] An asterisk (*) represents both nonessential elements and elements whose biological essentiality as yet remains unproved.

Additional correlations between biological essentiality and chemical periodicity are evident if the essential elements are arranged in a series of groups (table 2). When arranged in a series according to atomic number the essential elements form groups, of one to four members, which appear to bear definite numerical relations to each other. This is made plain by changes in the number of members of consecutive groups. The discrepancies in the number of group members of the elements having higher atomic numbers are due in all probability to blanks caused by the omission of essential elements not yet identified. The differences between the atomic numbers of the first members of these series groups also exhibit definite regularities, though here again unavoidable omissions cause irregularities in the values between groups of higher atomic number. Similarly, the numbers of elements omitted between consecutive groups of known essential elements are 3, 2, 1, 1, 3, 1, 10, and 10, respectively. Silicon has been included as an essential element because of its use for cell-wall construction by the Diatomaceae, though its essentiality has never been demonstrated and its omission increases the regularities of the table markedly. Two exceptions exist to the rule that the

initial element of each group has an odd atomic number. These are silicon (atomic number 14) and molybdenum (atomic number 42). It is not impossible, therefore, that silicon may eventually be determined to be nonessential and columbium essential. Another interesting feature of series-group arrangement is the differences in atomic number of the initial members of the positive-element groups. These differences are 10, 8, 6, 4, and 13 for consecutive groups. The difficulties with table 2 as a means of bringing out correlations are inherent in the use of series groups.

TABLE 2.—*The group periodicity of biologically essential elements as a function of atomic number*

Group No.	Symbols of elements, preceded by atomic numbers [1]	Members in indicated group	Difference between atomic numbers of first members of consecutive groups	Nonessential elements between consecutive groups of known essential elements
		Number		*Number*
1	(1,H)	1	------	------
2	(5, B)–(6, C)–(7, N)–(8,O)	4	4	3
3	(11,Na)–(12,Mg)	2	6	2
4	(14,Si)–(15,P)–(16,S)–(17,Cl)	4 [2](3)	3 [2](4)	1 [2](2)
5	(19,K)–(20,Ca)–(21, (Sc))	3	5 [2](4)	1
6	(25,Mn)–(26,Fe)–(27,Co)	3	6	3
7	(29,Cu)–(30,Zn)–(31,Ga)	3	4	1
8	(42,Mo)	1	13	10
9	[3]*	?	?	?
10	(53,I)	1	11	10

[1] Atomic numbers in each group are consecutive.
[2] Value if silicon is considered unessential.
[3] The asterisk (*) represents a group of elements whose biological essentiality as yet remains unproved.

Correlations between essentiality and the inner physics of the atom can be shown best, however, through the tabulation of the 92 elements on the basis of their transition subshells, or the subshells in which the electron numbers of the atoms have undergone a *regular* change in the formation of the elements. These are the electrons that determine to a large extent the optical and chemical properties of the elements of which they form a part. Table 3 presents the chemical elements arranged, on the basis of transition subshells, according to shell, subshell, atomic number, and "rank," to bring out the relation of atomic structure to the property of biological essentiality. The atomic data for the individual elements in the normal state are those given by White (*10*). The shells into which the electrons are arranged about the nucleus of the atom are designated as n=1, n=2, n=3, n=4, n=5, n=6, and n=7 from the nucleus outward. Each shell may comprise one or more subshells, designated as l=0, l=1, l=2, and l=3, also numbered from the center. The symbol for each element is preceded by the atomic number of the element. The numbers 1 to 14 at the heads of columns indicate the order in which the elements occur in each subshell row when arranged according to the magnitude of their atomic numbers. For these values the term "rank" will be used. This arrangement is more fundamental than that of the standard chemical periodic table inasmuch as spatial relations as well as number of electrons in the atoms are considered.

TABLE 3.—*The chemical elements arranged on the basis of the subshell undergoing regular change in electron number* [a]

Shell No.	Subshell No.	Atomic numbers and symbols of chemical elements [a] in indicated rank [b]														Elements per shell		Known essential elements per shell		Elements per shell according to Rydberg	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	Number	Factors	Number	Factors	Number	Factors
n=1	l=0	*1, H*	2, He													2	2×1^2	1	1^2	2	2×1^2
n=2	l=0	3, Li	4, Be													8	2×2^2	4	2^2	8	2×2^2
	l=1	*5, B*	*6, C*	*7, N*	*8, O*	9, F	10, Ne														
n=3	l=0	*11, Na*	*12, Mg*													18	2×3^2	12	3×2^2	8	2×2^2
	l=1	13, Al	*14, Si*	*15, P*	*16, S*	*17, Cl*	18, A														
	l=2	*21, Sc*	22, Ti	23, V	24, Cr	*25, Mn*	*26, Fe*	*27, Co*	*28, Ni*	*29, Cu*	*30, Zn*										
n=4	l=0	*19, K*	*20, Ca*													32	2×4^2	4	2^2	18	2×3^2
	l=1	*31, Ga*	32, Ge	33, As	34, Se	35, Br	36, Kr														
	l=2	39, Y	40, Zr	41, Cb	*42, Mo*	43, Ma	44, Ru	45 Rh	46, Pd	47, Ag	48, Cd										
	l=3	58, Ce	59, Pr	60, Nd	61, Il	62, Sm	63, Eu	64, Gd	65, Tb	66, Dy	67, Ho	68, Er	39, Tm	70, Yb	71, Lu						
n=5	l=0	37, Rb	38, Sr													18	2×3^2	1	1^2	18	2×3^2
	l=1	49, In	50, Sn	51, Sb	52, Te	*53, I*	54, Xe														
	l=2	57, La	72, Hf	73, Ta	74, W	75, Re	76, Os	77, Ir	78, Pt	79, Au	80, Hg										
	l=3																				
n=6	l=0	55, Cs	56, Ba													12	3×2^2	0	0	32	2×4^2
	l=1	81, Tl	82, Pb	83, Bi	84, Po	85	86, Rn														
	l=2	89, Ac	90, Th	91, Pa	92, U																
	l=3																				
n=7	l=0	87	88, Ra													2	2×1^2	0	0	6	2×3
	l=1																				
	l=2																				
	l=3																				
Total elements per rank		17	17	10	10	9	9	4	4	4	4	1	1	1	1						
Essential elements	l=0	H, Na, K	Mg, Ca																		
	l=1	B, Ga	C, Si	N, P	O, S	Cl, I															
	l=2	(Sc)	[c] *	*	Mo	Mn	Fe	Co	*	Cu	Zn										
	l=3	*																			
Known essential elements per rank		6	4	2	3	3	1	1	0	1	1										

[a] The chemical elements are indicated by their chemical symbols and are preceded by their atomic numbers. Italics indicate essential elements.
[b] The "rank" represents the relative order of the elements of each subshell when arranged according to atomic number.
[c] Asterisks (*) indicate elements the biological essentiality of which has not been proved.

At the bottom of table 3 is what may be regarded as a summation of these data with respect to the biologically essential elements. The five essential elements in subshell l=0 are all positive, and their number decreases from 3 in rank 1 to 2 in rank 2. The 10 essential elements formed by a change in subshell l=1 are arranged uniformly in pairs in ranks 1 to 5, inclusive. Gallium occupies a position among these negative elements, whereas in table 2 it fell among the positive ones. Finally, the l=2 subshell row with seven elements appears to contain but a single essential element in each rank. The three blank spaces may represent elements the essentiality of which has not yet been proved. No element essential for nutrition occurs in the l=3 row, which includes the rare earths only, though the essentiality of cerium,[5] particularly, is not definitely eliminated. The total number of known essential elements in successive shells forms a very uniform series, namely, 1, 4, 12, 4, 1, 0, and 0, reckoned from the nucleus to the periphery of the atom. Sodium, calcium, boron, (gallium), chlorine, and iodine—forming a group of essential elements dispensed with by certain organisms—fall into terminal ranks of the subshells. The segregation of the essential elements into positive and negative groups and the progressive decrease in the number of such elements per rank in successive subshells, together with the uniformity in number of elements per rank in each subshell, cannot be attributed to chance. These regularities, rather, must be interpreted as an expression of the interplay of ordered forces having their seat in the mechanism of atomic structure.

The objection might be raised that the use of table 3, and particularly of the property termed the "rank," is unacceptable because of its wide departure from the arrangement of the Mendelejeff chemical periodic table. Nevertheless, this table, though of greater complexity, also serves to bring out the effects of position and number of electrons on the chemical and physical properties of the elements as well as or better than the standard table. Moreover, the places into which the elements fall are definite and without equivocation. Subshells l=0 and l=1 seem to be the positions particularly associated with the possession of metalloid properties, a characteristic that increases with the rank until the gaseous state is reached. Shell position, however, is also of some effect in this respect. Two of the series of elements afford a complete illustration of the Rydberg rule for increases in atomic number of homologous elements. Beginning with hydrogen, the elements of subshell l=0 in rank 1 increase in atomic number in successive amounts of 2, 8, 8, 18, 18, and 32. The same is true for the elements of subshell l=0 in rank number 2 beginning with helium. Other correlations are readily apparent. It would not be surprising, however, if certain discrepancies exist, since only three of the quanta of the atom and the rank form the basis of this classification. The characteristics of beryllium and magnesium would be expected to be less metallic. Ample chemical justification exists, nevertheless, for the use of this table to bring out the association between atomic structure and biological essentiality, and to employ it as a basis to demonstrate that the elements possessing the property of essentiality form not a random selection but a group correlated with respect to atomic structure.

[5] Cerium perhaps should occupy the position of hafnium, and the latter that after lutecium in the l=3 subshell.

As a matter of fact, the arrangement of table 3 is an improvement in certain respects over that of the standard table. Hydrogen finds a definite position, as do also the rare-earth metals and the members of group VIII. Changes in electron number in subshell l=3 effect little change in chemical properties of the elements as exemplified by the rare earths, which alone of all the elements occupy this position. The total number of elements in the shells differs from that of the Rydberg (*10*) series in being much more symmetrical. If each rank be divided into four subranks equivalent to positions in subshells l=0, l=1, l=2, and l=3, the elements arrange themselves into series quite similar to those of the familiar groups and subgroups of the standard periodic table. The relative positions of the elements are more in keeping with their chemical properties than in the standard table, however. Another difference lies in the number of ranks required to bring about a periodic change in properties of the elements. In subshell l=0, the equivalent of 2 elements, in subshell l=1, the equivalent of 6 elements, and in subshell l=2, the equivalent of 10 elements is required. That is, the periodic recurrence of properties with increase in atomic number differs uniformly with the transition subshells in the ratio of 2:6:10; or, if the rare-earth elements are included, the series is 2:6:10:14. Chemical valence is associated with the subrank and shows far fewer anomalies than in the Mendelejeff table. The number of elements per rank also forms an interesting series, namely, 17, 17, 10, 10, 9 9, 4, 4, 4, 4, 1, 1, 1, and 1. The pairing evident in this series is in accordance with the pairing shown by all the 92 elements and by the majority of the essential elements in this table. The factors for this series are 4^2+1, 3^2+1, 3^2+0, 2^2+0, and 1^2+0. Though its significance is unknown to the writer, its regularity and the uniformity of pairing in the values is believed to have a basis in atomic structure and serves to strengthen the belief that the correlations between biological essentiality and atomic structure based on the ranks of table 3 have an objective existence.

DISCUSSION

The deductions feasible on the basis of the correlations between atomic structure and essentiality with respect to the total number of essential elements and of their identity are vague, largely negative in character, and debatable. It appears probable from the data of table 1 that at least two, and perhaps three, additional elements will be found to be essential. These, it is assumed, would occur singly in groups IV and V and perhaps in group VIII. The data of table 2 are almost too ambiguous for prognostication, though the nonessentiality of silicon and the essentiality of columbium are indicated. Consideration of the data in table 3 would indicate to the writer that no more elements of shells n=1, n=2, and n=3 will be found essential; nor, if greatest weight is placed on the apparent regularity of the series of the total number of essential elements per shell, are any to be found in the other shells. However, the nonessentiality of silicon would indicate an unidentified essential element in shell n=3. The irregularities in the series formed by the total number of known essential elements per rank and the vacancies in the l=2 subshell row of essential elements may perhaps indicate otherwise. Mathematically, it would be practicable to fill the vacancies in subshell l=2 without seriously affecting

the uniformity of the series of total essential elements per shell, though an alteration would be necessary.

A thorough understanding of factors concerned should permit foreknowledge of experimental results. Nevertheless, attempted predictions in scientific investigations, as elsewhere, have been found extremely hazardous and are to be avoided as of dubious value, unless strongly justified as in this case. Search for the essential trace elements in the past has largely been purely empirical and directed almost by chance. The publication of these tables will, it is hoped, act as a beneficial directive influence, since a direct objective is now available that makes feasible the partial correlation of the data on the essential elements. It should not be forgotten, however, that no single organism seems to require all (22 or more) essential elements. This situation may be purely illusory and depend on low experimental precision, but it is probably associated with broad evolutionary trends that include gain or loss of function. The belief of the writer in the existence of only a few other as yet unidentified essential elements may therefore need considerable revision on the basis of experience with organisms other than *Aspergillus*. The writer is also convinced that eventually an even closer correlation between essentiality and atomic structure will be demonstrated by the atomic physicist through the mathematically complex quantum mechanics of the atom.

SUMMARY

Correlations were found between atomic structure and biological essentiality of the chemical elements that would indicate that the essential elements are closely correlated with respect to atomic structure and their distribution among the nonessential elements. Tentative, though vague and debatable, deductions are feasible with respect to the number and identity of the essential elements as yet unknown. A suggested form of chemical periodic table based on shell and subshell of transition, atomic number, and rank, is superior in certain respects to the standard table. Moreover, this arrangement of the chemical elements makes possible the correlation of atomic structure with the property of biological essentiality.

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LABORATORY STUDIES ON TETRASTICHUS XANTHOMELAENAE ROND. AND TETRASTICHUS SP., TWO HYMENOPTEROUS EGG PARASITES OF THE ELM LEAF BEETLE [1] [2]

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INTRODUCTION

Several attempts have been made to establish *Tetrastichus xanthomelaenae* Rond., an important egg parasite of the elm leaf beetle (*Galerucella xanthomelaena* (Schr.)), in the United States. The first shipments were made from France in 1908,[3] and up to 1932 occasional attempts were made to establish this species in the New England States. In 1932 importations on a larger scale were undertaken, and parasitized eggs were received from both France and Austria. These shipments were made early in the season in order to give the parasites access, upon their liberation in the United States, to the greatest possible number of elm leaf beetle eggs. Emergence of parasites began soon after the parasitized eggs arrived at the Melrose Highlands laboratory.

After many of the parasites had emerged, it was discovered that two species of *Tetrastichus* were present. One was *T. xanthomelaenae* and the other an unidentified species. Separation of the living specimens of these two species was difficult and was not considered to be practical in view of the large quantity of material received. It seemed advisable to hold all material in the laboratory until the status of the new species could be determined.

The percentage of *Tetrastichus* sp. occurring in the imported material has been low. A sample of 600 adults from Oberpullendorf, Austria, contained 29 *Tetrastichus* sp., or 4.8 percent. In a sample of 500 adults from Hyères, France, there were 17 *Tetrastichus* sp., or only 3.4 percent.

Apparently *Tetrastichus* sp. has always been present in much smaller numbers than *T. xanthomelaenae*. Marchal [4] made a number of observations on *T. xanthomelaenae* but did not encounter the new species in his work. In another paper,[5] in which he described *T. xanthomelaenae*, Marchal mentioned another parasite reported as attacking eggs of *Galerucella xanthomelaena*, but the record of the exact host is questionable and the brief notes on the parasite indicate that it was not *Tetrastichus* sp.

[1] Received for publication June 17, 1938; issued December, 1938.

[2] This study was conducted at the Melrose Highlands, Mass., laboratory of the Bureau of Entomology and Plant Quarantine during 1932 and 1935. The writer is indebted to C. W. Collins, in charge of the laboratory previous to 1935, and to R. C. Brown, in charge during 1935, for making these investigations possible, and to P. B. Dowden for helpful advice.

[3] HOWARD, L. O. THE IMPORTATION OF TETRASTICHUS XANTHOMELAENAE (ROND.). Jour. Econ. Ent. 1 281–289, illus. 1908.

[4] MARCHAL, P. OBSERVATIONS BIOLOGIQUES SUR UN PARASITE DE LA GALÉRUQUE DE L'ORME, LE TETRASTICHUS XANTHOMELAENAE (ROND.) (HYMÉN.). Bull. Soc. Ent. France 1905: 64–68. 1905.

[5] MARCHAL, P. IDENTIFICATION DU PARASITE DES OEUFS DE LA GALÉRUQUE DE L'ORME, TETRASTICHUS XANTHOMELAENAE (ROND.) (HYMÉN.). Bull. Soc. Ent. France 1905: 81–83, illus. 1905.

Parker [6] figured the egg, the first-instar larva, and the full-grown larva of *Tetrastichus* sp., but he reported it as *T. xanthomelaenae*. This is not surprising, for prior to 1932 only one species was known to attack the eggs of the elm leaf beetle.

EXPERIMENTS ON THE TWO PARASITES WHEN OCCURRING SEPARATELY AND WHEN IN COMPETITION

Experiments were undertaken to determine the status of each species of parasite when occurring separately, the effect that one would have upon the efficiency of the other when they were operating together, and whether either species acted as a secondary parasite upon the other species. For this purpose it was necessary to conduct several series simultaneously. In all the experiments the development was observed through daily dissections of a portion of the exposed material.

In the first series of experiments each species of parasite was reared separately from egg to adult on clusters of elm leaf beetle eggs collected from several localities in Massachusetts, and the life cycles and the principal habits of the two species were studied. *Tetrastichus xanthomelaenae* oviposited freely in the elm leaf beetle eggs, dissections showing that each egg contained from one to four eggs of the parasite. Only one individual, however, developed to maturity in a single host egg; the others succumbed in an early stage of development. The cause of the death of the excess young larvae where superparasitization occurred was not apparent. Adult males and females issued in about equal numbers from all the lots exposed. The average length of the developmental period from the time the eggs were laid until the adults issued was 16.9 days. The average life cycle of *Tetrastichus* sp. was found to be 17.5 days, or about 14 hours longer than that of *T. xanthomelaenae*. In other respects the development was the same in the two species.

In the second series of experiments several masses of elm leaf beetle eggs were exposed to a mixed lot of parasites containing equal numbers of mated females of each species. Dissections of a part of the material showed a high percentage of parasitization and considerable superparasitization. Frequently eggs and larvae of both species of parasites were found in the same host egg. Only one of the larvae developed to maturity, however. Sometimes it was one species and sometimes the other. Out of 200 dissected eggs that contained both species of larvae with only one larva living, there were 85 eggs in which the larvae of *Tetrastichus xanthomelaenae* remained alive and 115 in which the *Tetrastichus* sp. survived. The eggs that were allowed to develop to maturity produced 105 *T. xanthomelaenae* and 93 *Tetrastichus* sp. It is evident, therefore, that the chances of developing to maturity are about equal for the two species when they both attack at the same time.

In the third set of experiments egg masses were exposed first to one species of *Tetrastichus* and then to the other. Several egg masses were exposed to *T. xanthomelaenae* for 4 hours. This material was then divided into 10 lots, which were numbered from 1 to 10. Lot No. 1 was exposed to *Tetrastichus* sp. the day following exposure to

[6] PARKER, H. L. RECHERCHES SUR LES FORMES POST-EMBRYONNAIRES DES CHALCIDIENS. Ann. Soc Ent. France 93: [261]-379, illus. 1924.

T. xanthomelaenae, lot No. 2 the second day, and so on until the sixth day, when the parasites refused to oviposit in the parasitized eggs. Dissections of a number of samples showed that oviposition by *Tetrastichus* sp. in the parasitized eggs decreased after the second day and only a few of those offered on the fifth day were attacked. There were a few cases in which the *Tetrastichus* sp. survived where from 1 to 2 days intervened between the periods of attack. Where there was an interval of more than 2 days, *T. xanthomelaenae* survived in all cases observed.

In the experiments in which the order of attack by the parasites was reversed, the results were similar in that the species attacking first survived in much the greater numbers. Neither species, therefore, acted as a secondary parasite on the other.

CHARACTERS FOR SEPARATING THE EARLY STAGES AND ADULTS OF TETRASTICHUS XANTHOMELAENAE AND TETRASTICHUS SP.

Eggs and early-instar larvae of these two species of *Tetrastichus* may be readily separated, but no distinguishing characters have been found for separating the later instars or the pupae.

EGG

The egg of *Tetrastichus xanthomelaenae* (fig. 1, *D*) measures 0.23 mm in length and 0.075 mm at its greatest width. It is almost circular in cross section and slightly curved in shape. It is white and has a smooth surface. From the cephalic end, which is bluntly rounded, the egg thickens slightly for a short distance and then gradually tapers to a round caudal end.

The egg of *Tetrastichus* sp. (fig. 1, *B*) measures 0.22 mm in length and 0.06 mm at its greatest width. It is almost circular in cross section and slightly curved in shape. It is white and has a smooth, glistening surface. The cephalic end has a protuberance resembling a petiole. From this petiolar region the egg thickens abruptly to its greatest diameter, from which it tapers gradually to a blunt caudal end.

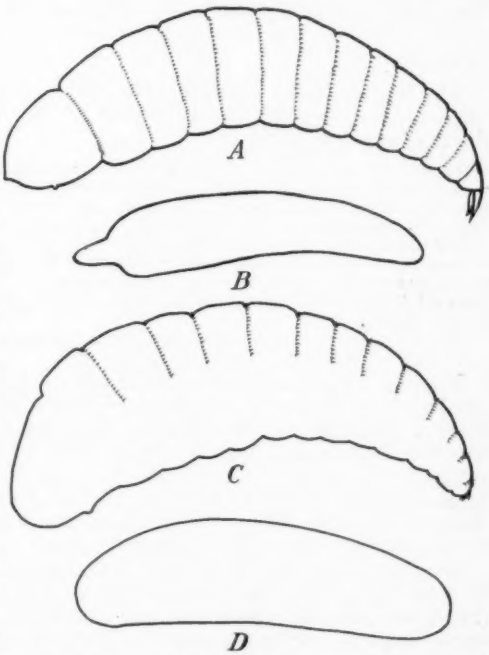

FIGURE 1.—*Tetrastichus* sp.: *A*, First-instar larva, and *B*, egg. *Tetrastichus xanthomelaenae*: *C*, First-instar larva, and *D*, egg.

LARVA

The first-instar larva of *Tetrastichus xanthomelaenae* (fig. 1, *C*), shortly after emerging from the egg, is 0.32 mm in length and 0.09 mm at its greatest width. The head is long and shaped like a thimble, but it is shorter and thicker than the head of *Tetrastichus* sp. There are 3 thoracic and 10 abdominal segments. The last segment is short and has a complete row of caudal hairs or spines, which are heavier than those on the other segments. On the posterior margins of each of the other segments there is a row of spines of about equal length extending across the dorsum and almost to the lateral margins. The young larva is hyaline, the midintestine being visible through the body walls.

The first-instar larva of *Tetrastichus* sp. (fig. 1, *A*), shortly after emerging from the egg, measures 0.37 mm in length and 0.09 mm at its greatest diameter. It is cylindrical in form, with diameter decreasing towards the posterior extremity. It has a long, thimble-shaped head and 3 thoracic and 10 abdominal segments. The last abdominal segment is terminated by two long, slightly curved hooks placed side by side. All segments except the last have rows of spines completely encircling the larva. The larva is hyaline.

ADULT

The characters for separating the adults of these two species of parasites, as described by B. D. Burks,[7] of the Illinois Natural History Survey, are as follows:

Tetrastichus xanthomelaenae (Rond.)—Thorax dark metallic blue-green, abdomen entirely black, with faint metallic luster; pedicel of antenna globose in both sexes; flagellum of the males bearing short setae; none longer than the third funicle segment; submarginal vein of forewing with one dorsal bristle.

Tetrastichus sp. "Y"—Thorax shining black, abdomen dark brown and yellow at base; pedicel of antenna slender, elongate, slightly enlarged at apex in both sexes; flagellum of male bearing long setae; those borne by the second funicle segment longer than the entire funicle; submarginal vein of forewing with two dorsal bristles.

LIBERATIONS AND RECOVERIES

Prior to 1932 from 2,000 to 2,500 adults of *Tetrastichus xanthomelaenae* had been liberated at various localities in New England and New Jersey. A large number of these were reared in the laboratory at Melrose Highlands, Mass.[8] From 1932 to 1935, inclusive, 35,234 adults were imported and liberated in the United States, as follows: Massachusetts, 8,445; Connecticut, 4,000; New Hampshire, 600; New York, 1,000; New Jersey, 2,484; District of Columbia, 3,950; Virginia, 2,750; Ohio, 5,000; and California, 7,005.

Several collections of elm leaf beetle eggs in Massachusetts and one in New Jersey have shown that the parasites reproduce in the field the same season in which they are liberated, but none have been recovered the following season. In 1933 collections of 449 egg masses from the sites of the 1932 liberations and in 1934 collections of 689 egg masses from the 1932–33 liberation points failed to produce a single parasite.

[7] Private communication.

[8] HOWARD, L. O., and FISKE, W. F. THE IMPORATION INTO THE UNITED STATES OF THE PARASITES OF THE GYPSY MOTH AND THE BROWN TAIL MOTH: A REPORT OF PROGRESS, WITH SOME CONSIDERATION OF PREVIOUS AND RECURRENT EFFORTS OF THIS KIND. U. S. Bur. Ent. Bull. 91, 344 pp., illus. 1911.

SUMMARY

In the course of attempts to establish the elm leaf beetle parasite *Tetrastichus xanthomelaenae* Rond. in the United States, another, unidentified, species of *Tetrastichus* was discovered in the imported material. A study of the two species was therefore undertaken in the laboratory to observe their development and parasitic habits, both when each species occurred separately and when the two were in competition. The characters distinguishing the two species were also determined.

The development was found to be similar in the two species, except that the life cycle of *Tetrastichus* sp. was slightly longer than that of *T. xanthomelaenae*. When superparasitism or multiple parasitism occurred, all but one larva in each host egg died in the early instars. Where the host was attacked by both species, the one attacking first had the better chance of surviving. Both species are therefore primary parasites.

The eggs and young larvae of the two parasites have definite characters by which they can be readily separated, but no distinguishing characters were found for the later instars or the pupae.

In all importations the percentages of adult *Tetrastichus* sp. emerging from the beetle eggs were low, never exceeding 5 percent.

EFFECT OF EXTERIOR TEMPERATURE UPON PRESS FLUID, SHEAR FORCE, AND COOKING LOSSES OF ROASTED BEEF AND PORK MUSCLES [1]

By ALICE M. CHILD, *associate professor of home economics*, and MARY J. SATORIUS, *instructor in home economics, Minnesota Agricultural Experiment Station* [2]

INTRODUCTION

In meat cookery, methods must be worked out by which palatability can best be retained and developed. Palatability is in large measure dependent upon the structure of the muscle tissue. The present work was undertaken to study the effect of exterior or oven temperature upon structure of meat when the two following mechanical devices were employed: The pressometer, used by Child and Fogarty (*4*),[3] which measures press fluid (defined as fluid consisting of moisture plus the soluble material plus the colloidal fraction pressed from muscle by the pressometer), and the Minnesota modification of the Warner-Bratzler shear-force apparatus which measures the force necessary to shear a sample of meat of given dimensions.

The study covered the effect of oven or exterior temperature upon press fluid, shear force, and cooking losses during the roasting of the following muscles: (1) Semitendinosus beef muscle heated to an internal temperature of 58° C. at oven temperatures of 125°, 150°, 175°, and 200°; (2) longissimus dorsi beef muscle heated to an internal temperature of 58° at constant oven temperatures of 150° and 200°, and at 150° after searing at 260°; and (3) longissimus dorsi pork muscle heated to an internal temperature of 84° at constant oven temperatures of 125°, 150°, and 175°, and at 150° after searing at 260°.

MATERIALS AND METHODS

Beef of high medium to good grade which had been ripened 12 days at 2° to 3° C. was obtained from a local packing plant. The semitendinosus or "eye" muscle from the bottom round had been found homogeneous by Child and Fogarty (*4*) so that two comparable roasts, weighing about 1.5 pounds each, could be obtained from each muscle, giving four comparable roasts from one animal. A series of 12 animals was studied, 48 roasts in all. The longissimus dorsi muscle had not yet been studied for homogeneity at the time of this experiment. Thus, from a series of 18 animals pairs of seventh-eighth standing ribs, weighing about 7 pounds each, were used for one comparison of external temperatures, and ninth-tenth ribs, weighing about 6 pounds each, were used for another, a total of 72 beef rib roasts.

Pork was obtained through the local packing plant and was ripened 4 days at 2° to 3° C. The longissimus dorsi muscle from center pork

[1] Received for publication February 1, 1938; issued December, 1938. Scientific Journal series, paper No. 580, Minnesota Agricultural Experiment Station.

[2] The authors gratefully acknowledge their indebtedness to George Steinacher for technical assistance in constructing the apparatus shown in figure 1, and to L. W. Neubauer, Agricultural Engineering Department, for the drawing.

[3] Italic numbers in parentheses refer to Literature Cited, p. 871.

loin containing four thoracic and four lumbar vertebrae had been found to be homogeneous,[4] so that the cut could be halved between the thoracic and lumbar vertebrae to give two comparable roasts weighing 1.8 pounds each. Thus, four comparable roasts from each of 12 animals or a total of 48 roasts were used.

ROASTING AND SAMPLING MEAT

All meat was cooked in electric laboratory ovens, controlled to 2° F. with regulators, by methods recommended by the cooking committee [5] of the cooperative meat investigation committee. Cooking data, i. e., losses, cooking time, and temperature rise after removal from the oven were recorded.

The adipose covering of the semitendinosus muscle was removed and the four comparable roasts were cooked to 58° C. at oven temperatures of 125°, 150°, 175°, and 200°. The meat was cooled to 40° for sampling. To better control biological variation the order of roasting was rotated so that one cut was not always cooked at the same temperature.

The seventh-eighth and ninth-tenth beef ribs were cooked to 58° C. One of each pair of seventh-eighth ribs was cooked at 150° constant temperature and compared to the other one which was cooked at 150° after searing for 20 minutes at 260°. One of each pair of ninth-tenth ribs was cooked at 150° and compared to the other one cooked at 200°. The meat was cooled to 40° for sampling.

The four comparable roasts from center pork loin were cooked to 84° C. at constant oven temperatures of 125°, 150°, and 175°, and at 150° after searing for 20 minutes at 260°. The roasts were rotated as for the semitendinosus muscle. The meat was cooled to 80° for sampling.

After cooling, the fat and bone on the roast were removed and the muscle was halved through the thermometer hole perpendicular to the muscle fibers. A slice 1.25 cm thick was cut for press-fluid determinations, and from this two samples, on either side of the thermometer hole, were taken parallel to the muscle fibers with a borer 1.25 cm in diameter. The other half of the roast was used for tenderness determinations and from it two samples, about 3.8 cm long, were taken parallel to the muscle fibers with a borer 2.54 cm in diameter.

PRESS-FLUID DETERMINATION

The two samples of meat cut for press-fluid determinations were weighed to the nearest tenth of a milligram. Each sample, weighing about 1.5 g, was wrapped in filter cloth and pressed 10 minutes under a pressure of 250 pounds by the pressometer, which was standardized by Child and Baldelli (*3*). The difference in weight of the meat before and after pressing was referred to as the weight of the press fluid.

SHEAR-FORCE DETERMINATION

A modification of the Warner-Bratzler [6] shear-force apparatus was used to measure shear force, and figure 1 shows its mechanism. Each

[4] CHILD, ALICE M., and SATORIUS, MARY J. A STUDY OF SAMPLING FOR MEAT RESEARCH. Unpublished data, Minnesota Experiment Station. 1937.

[5] ALEXANDER, LUCY M., CLARK, N. G., and HOWE, P. E. METHODS OF COOKING AND TESTING MEAT FOR PALATABILITY. Supplement to National Project Cooperative Meat Investigations. U. S. Dept. Agr., Bur. Home Econ. and Bur. Anim. Indus. 36 pp., illus. Revised February 1933. Mimeographed.

[6] BRATZLER, T. J. MEASURING THE TENDERNESS OF MEAT BY MEANS OF A MECHANICAL SHEAR. Unpublished data, Kansas State College. 1932.

sample cut for shear-force determination was inserted in a triangular opening in a dull blade 1 mm in thickness. Shearing bars were started downward at the rate of 23.5 cm per minute by an automatic switch and electric motor and the pounds of force necessary to shear the

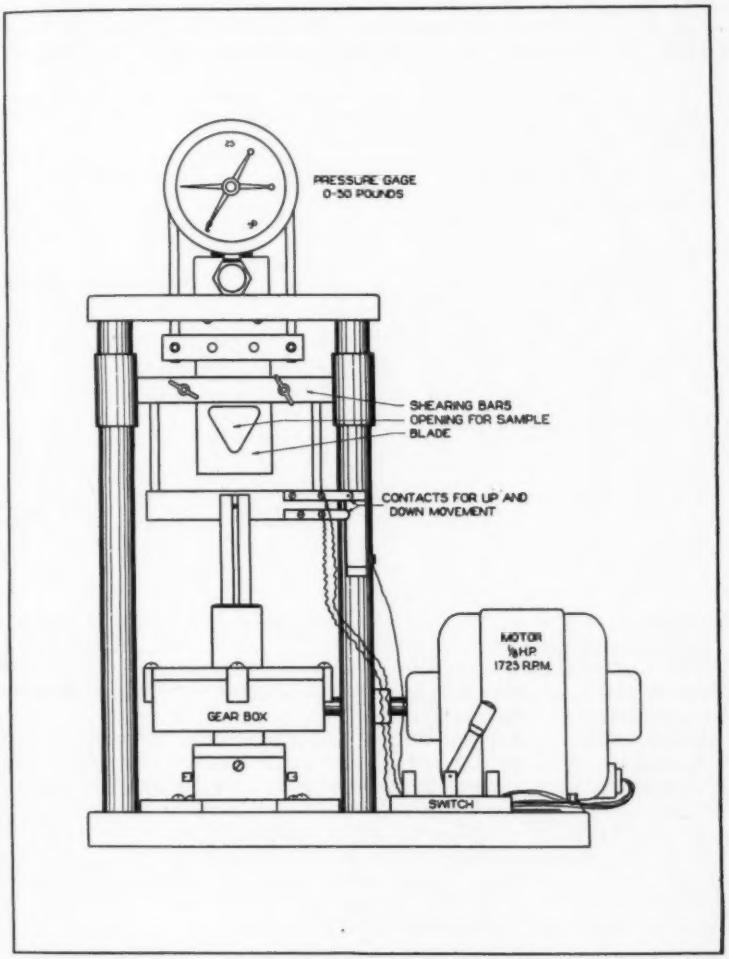

FIGURE 1.—Diagram of shear-force apparatus, showing detail of working mechanism.

meat were recorded to the nearest one-fourth pound on the gauge. Three determinations, one at the center and one at the center of each half, were made on each of the two samples; the six readings were averaged for each roast.

EXPERIMENTAL DATA

SEMITENDINOSUS MUSCLE

The roasts cooked to 58° C. at 125°, 175°, and 200° were compared to those cooked at 150°, since this oven temperature has been generally accepted. Press fluid and shear force were not significantly affected by exterior temperature, as shown by the Fisher (7) *t* test (table 1). The values for *t* did not exceed 1.3, which has a corres-

TABLE 1.—*Percentage press fluid, total losses, and pounds of shear force for semitendinosus beef muscle heated to an internal temperature of 58° C. at oven temperatures of 125°, 150°, 175°, and 200°*

Series No.	Press fluid				Total losses				Shear force			
	125° C.	150° C.	175° C.	200° C.	125° C.	150° C.	175° C.	200° C.	125° C.	150° C.	175° C.	200° C.
	Per-cent (1)	*Per-cent* (2)	*Per-cent* (3)	*Per-cent* (4)	*Per-cent* (1)	*Per-cent* (2)	*Per-cent* (3)	*Per-cent* (4)	*Pounds* (1)	*Pounds* (2)	*Pounds* (3)	*Pounds* (4)
1	55.63	53.29	56.05	59.27	14.84	14.51	16.78	19.49	11.6	12.1	14.0	17.7
2	54.33	52.93	55.15	54.13	14.93	14.19	13.62	16.16	15.9	13.1	11.1	13.5
3	57.01	55.89	55.43	54.93	12.47	14.78	16.32	18.61	17.5	14.5	15.9	14.5
4	52.91	54.61	53.45	55.64	14.54	15.83	18.42	17.01	12.2	12.5	11.7	10.9
5	56.92	57.53	55.25	54.57	14.53	14.85	16.41	18.12	10.9	12.3	15.3	15.1
6	56.11	54.18	53.72	49.68	14.02	14.66	16.84	20.57	11.6	9.1	13.7	16.7
7	57.12	58.08	55.96	57.97	13.14	14.81	20.80	19.22	14.9	12.9	12.9	11.5
8	56.83	56.51	54.35	52.31	15.37	16.25	20.26	19.65	7.5	10.5	11.9	12.3
9	54.43	54.89	54.73	57.91	11.06	12.01	18.95	16.97	10.2	10.6	11.3	8.3
10	52.89	54.23	55.14	53.19	13.68	13.94	12.92	17.09	16.3	12.3	10.5	17.1
11	51.29	53.91	56.36	55.91	14.04	14.04	14.39	16.46	15.1	15.9	11.3	10.9
12	54.64	55.18	56.27	55.73	14.55	14.75	14.57	17.66	11.7	10.7	10.7	14.7
Mean	55.01	55.10	55.15	55.10	13.93	14.55	16.69	18.08	12.9	12.2	12.5	13.6
	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)
	0.21		0.10	0.00	2.50		2.97	8.97	1.21		0.45	1.30
P	>.56		>.56	>.56	.03		.01	<.01	.25		>.56	.22

ponding value of $P=0.22$. This value is above the 5-percent level for significance or the 1-percent level for high significance. Total losses, however, were found to increase with each increment in exterior temperature; the increase was due to evaporation losses only. The values for *t* ranged from 2.50 to 8.97.

It has furthermore been shown [7] that when semitendinosus muscle is heated to 58° C. by dry heat, by cooking in a covered pan and by adding water in a covered pan, press fluid and shear force are not affected but cooking losses are increased by the moist heat.

A correlation of −0.05 was found between press fluid and total cooking losses for semitendinosus muscle heated to 58° C., a value which is not significant. Child and Fogarty (4) also found an insignificant correlation of −0.18 between press fluid and total losses for semitendinosus muscle heated to 58°.

STANDING BEEF RIBS

Press fluid did not vary significantly among beef ribs heated to 58°C. at constant temperatures of 150° and 200° and at 150° after searing at 260° (table 2). Values for *t* were not higher than 0.93, which give values for *P* far above the 5-percent level of significance. Searing did not affect shear force, but a significantly greater number of pounds

[7] NIENOW, I. EFFECT OF MOIST HEAT ON SEMITENDINOSUS MUSCLE. Unpublished data, Minnesota Agricultural Experiment Station. 1937.

of force were required to shear the ribs heated at 200° than those heated at 150°, a value for t of 2.99 being obtained. Cover (*6*) found beef ribs cooked well-done at 225° less tender when subjectively judged than those cooked at 125°, a finding which she thought due to the short cooking period at 225°.

TABLE 2.—*Percentage press fluid, total losses, and pounds of shear force for standing beef ribs heated to an internal temperature of 58° C. at constant oven temperatures of 150° and 200°, and at 150° following searing at 260°*

Series No.	Press fluid				Total losses				Shear force			
	Seared,[1] 150° C.	Constant, 150° C.	200° C.	150° C.	Seared,[1] 150° C.	Constant, 150° C.	200° C.	150° C.	Seared,[1] 150° C.	Constant, 150° C.	200 °C.	150° C.
	Percent (1)	*Percent* (2)	*Percent* (1)	*Percent* (2)	*Percent* (1)	*Percent* (2)	*Percent* (1)	*Percent* (2)	*Pounds* (1)	*Pounds* (2)	*Pounds* (1)	*Pounds* (2)
1	49.59	49.89	50.93	48.22	14.83	12.42	15.32	11.91	19.0	16.0	19.7	22.6
2	47.26	46.89	48.90	41.76			22.52	15.41	21.8	22.4	20.4	18.1
3	46.95	50.95	47.88	46.67	16.43	14.65	25.88	15.48	21.1	24.5	22.7	26.0
4	47.20	49.93	50.36	50.60	19.22	15.17	25.60	17.40	18.2	19.4	22.0	16.4
5	50.17	54.80	48.72	56.51	15.93	11.31	24.31	11.55	22.3	20.1	20.0	18.5
6	48.77	50.15	46.83	49.08	19.16	15.84	32.87	17.65	27.3	21.6	27.4	22.6
7	57.61	49.00	45.91	53.41	16.01	17.26	29.60	18.50	24.0	19.3	20.2	19.3
8	50.73	51.31	47.83	47.73	13.39	14.65	18.55	12.24	18.9	20.2	25.7	17.6
9	44.95	46.59	48.42	48.97	16.84	12.70	21.11	13.85	30.0	31.3	17.3	11.7
10	48.85	44.95	45.48	46.92	15.76	15.74	18.45	11.36	21.5	25.7	29.1	21.2
11	54.49	54.67	52.35	49.52	14.37	12.57	16.75	10.34	22.2	20.5	16.8	14.8
12	47.95	51.49	50.37	50.07	15.23	13.53	19.10	12.63	21.5	25.5	16.9	21.7
13	50.24	49.29	46.22	49.26	16.81	15.35	22.93	14.14	30.5	31.4	31.4	17.0
14	43.81	47.76	48.52	51.41	19.28	16.22	19.71	13.60	25.1	28.0	24.0	17.7
15	43.63	46.71	51.79	49.41	16.96	15.63	17.21	12.62	20.9	22.3	34.1	21.3
16	42.24	48.08	48.70	50.33	16.49	16.06	21.99	11.10	22.6	21.2	23.3	26.1
17	45.79	41.37	49.97	53.73	17.19	15.67			20.8	21.4	22.7	16.7
18	49.65	47.31	51.15	51.03	16.69	14.02	19.44	13.49	38.8	32.1	27.8	24.8
Mean	48.33	48.95	48.91	49.70	16.50	14.64	21.84	13.72	23.7	23.5	23.4	19.7
	(1) and (2)		(1) and (2)		(1) and (2)		(1) and (2)		(1) and (2)		(1) and (2)	
t	0.72		0.93		4.47		10.96		0.24		2.99	
P	.48		.37		<.01		<.01		>.56		<.01	

[1] 20 minutes at 260° C.

Cooking losses were increased both by a temperature of 200° C. and by initial searing (table 2), values for t of 10.96 and 4.47, respectively, being found. It has also been found by Cline (*5*) that beef cooked to 58° C. suffers more losses when cooked at high oven temperatures and when seared than at low constant temperatures and by Alexander (*1*) that high oven temperatures increase losses.

Furthermore, it has been shown [8] that beef ribs cooked to 58° C. in covered and uncovered pans do not differ in press fluid or shear force but cooking losses are increased by covering.

PORK LOINS

The pork loins cooked to 84° C. at constant temperatures of 125° and 175°, and at 150° following searing at 260°, were compared to those cooked at 150°. Press fluid of pork loin was not affected by different oven temperatures (table 3), for values of t did not exceed 1.67, which corresponds to $P=0.12$, an insignificant value. Shear force was not affected by oven temperature except that less force was required to shear the loins cooked at 125° than those cooked at 150°, a value for t

[8] NIENOW, I. See footnote 7, preceding page.

of 3.50 being obtained. The long cooking period of 138 minutes per pound at 125° as compared to 83 minutes at 150° may explain this difference. Only 57 minutes per pound were required at 175° and 62 at 150° following searing at 260°.

Total cooking losses of pork loin heated to 84° C. were not affected by constant exterior temperatures of 125°, 150°, and 175°, or by a temperature of 150° following searing, for all values of t were 0.76 or below. Alexander (*2*) found that cooking losses were not affected by oven temperatures when leg of lamb was cooked to 84°, but when cooked to 76° losses were increased by higher oven temperatures.

A significant correlation, −0.45, was found between press fluid and cooking losses of pork loin cooked to 84° C.

TABLE 3.—*Percentage press fluid, total losses, and pounds of shear force for pork loins heated to an internal temperature of 84° C. at constant oven temperatures of 125°, 150°, 175°, and at 150° following searing at 260°*

Series No.	Press fluid				Total losses				Shear force			
	125° C.	150° C.	175° C.	Seared [1] 150° C.	125° C.	150° C.	175° C.	Seared [1] 150° C.	125° C.	150° C.	175°C.	Seared [1] 150° C.
	Pct. (1)	*Pct.* (2)	*Pct.* (3)	*Pct.* (4)	*Pct.* (1)	*Pct.* (2)	*Pct.* (3)	*Pct.* (4)	*Lbs.* (1)	*Lbs.* (2)	*Lbs.* (3)	*Lbs.* (4)
1	38.11	42.44	45.45	51.44	20.42	22.35	23.60	17.53	6.1	9.6	6.5	9.3
2	40.59	40.67	49.23	43.85	21.24	25.56	22.26	25.44	6.5	7.6	7.6	9.5
3	45.39	44.85	47.43	50.21	20.66	26.53	20.70	22.25	8.1	8.3	8.5	8.5
4	35.96	41.93	42.22	38.78	21.57	20.41	22.17	26.47	9.3	10.3	10.6	12.0
5	42.33	43.12	42.14	49.21	17.93	20.15	22.50	18.81	5.5	10.1	8.6	8.1
6	29.58	48.48	38.03	42.92	26.13	23.51	25.64	22.50	5.6	8.0	8.5	8.5
7	37.59	34.60	41.18	41.44	26.50	25.53	24.76	24.02	6.5	8.5	6.3	7.6
8	39.85	37.54	39.93	37.05	18.66	27.06	27.91	25.85	6.5	9.8	9.0	13.0
9	42.98	44.75	47.55	40.89	29.22	24.32	23.35	26.81	4.3	4.3	7.8	6.8
10	33.48	38.18	34.97	39.46	31.13	20.93	22.61	20.45	6.1	7.5	7.1	6.6
11	44.06	47.72	37.67	44.06	24.52	18.36	22.39	23.71	7.8	7.5	6.6	7.6
12	45.51	44.78	44.06	38.22	21.56	17.12	21.78	23.83	6.3	6.3	7.0	8.8
Mean	39.62	42.42	42.49	43.13	23.29	22.65	23.31	23.14	6.5	8.1	7.8	8.9
	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)
t	1.67	------	0.04	0.46	0.41	------	0.76	0.44	3.50	------	0.64	1.50
P	.12	------	>.56	>.56	>.56	------	.46	>.56	<.01	------	.53	.16

[1] 20 minutes at 260° C.

SUMMARY AND CONCLUSIONS

Semitendinosus beef muscle heated to an internal temperature of 58° C. at oven temperatures of 125°, 150°, 175°, and 200° did not differ in press fluid or shear force. Cooking losses were increased with increased exterior temperature because of losses from evaporation.

Standing beef ribs heated to an internal temperature of 58° C. at constant oven temperatures of 150° and 200°, and at 150° following searing at 260°, showed no differences in press fluid or shear force except that more force was required to shear those cooked at 200° than those cooked at 150°. Cooking losses were greater at 200° and at 150° following searing than at 150° constant temperature.

Pork loin cooked to an internal temperature of 84° C. at constant oven temperatures of 125°, 150°, and 175°, and at 150° following searing at 260° did not differ in press fluid or shear force except that the loin cooked at 125° required less force to shear than that cooked at 150°. Cooking losses were not affected by exterior temperature.

From the data obtained the following conclusions may be drawn:

Exterior or oven temperatures of 125° to 200° C. do not affect press fluid of roasted meat, and only at the extreme ends of the temperature range is shear force affected. More pounds of force may be required to shear roasts cooked at temperatures of 200° or higher, and fewer pounds of force to shear those cooked at temperatures of 125° or lower. However, at the low temperatures, the cooking time is very long, especially for well-done meat.

High exterior temperatures and searing increase cooking losses in rare meat but not in well-done meat.

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